

FINAL REPORT

Bioconcentration Study of FRD903 with Carp

(Study No. A080560)

Submitted to :

DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.

Prepared by :

Mitsubishi Chemical Medience Corporation

June 26, 2009

**Bioconcentration Study of FRD903
with Carp**

(English version)

Sponsor DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.
Study Title Bioconcentration Study of FRD903 with Carp
Study No. A080560

This report is the English version of the original, which was written in Japanese. We, the undersigned, hereby declare that this version faithfully reflects the original report to the best of our knowledge.

Translated and Approved by

Hirokazu Kobayashi Date: July 15, 2009
Hirokazu KOBAYASHI, M.Sc.
Study Director
Yokohama Laboratory, Toxicological Science Division, Medi-Chem Segment,
Mitsubishi Chemical Medience Corporation

Compliance with the GLP Standards

Yokohama Laboratory,
Mitsubishi Chemical Medience Corporation

Sponsor DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.
Study Title Bioconcentration Study of FRD903 with Carp
Study No. A080560

I hereby certify that this study was carried out in accordance with its protocol and with our standard operating procedures, and that the reported results reflect accurately the raw data of the study.

The study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) standards concerning the test facility in which the study of new chemical substance is performed (Yakushokuhatsu No.1121003, Heisei 15.11.17 Seikyoku No.3, Kanpokiatsu No.031121004, November 21, 2003; the latest revision, July 4, 2008)

Study Director

Hirokazu KOBAYASHI, M.Sc.

Sealed date: June 26, 2009

Quality Assurance Statement

Yokohama Laboratory,
Toxicological Science Division Medi-Chem Business Segment
Mitsubishi Chemical Medience Corporation

Sponsor DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.
Study Title Bioconcentration Study of FRD903 with Carp
Study No. A080560

I hereby certify that this study was conducted in compliance with the following GLP, and that the final report reflects accurately the raw data of the study.

GLP Standards concerning the test facility in which the study of new chemical substance is performed (Yakushokuhatsu No.1121003, Heisei 15.11.17 Seikyoku No.3, Kanpokiatsu No.031121004, November 21, 2003; the latest revision, July 4, 2008).

The phase of study inspected and their dates, and the dates inspection results were reported to management and the Study Director are listed below.

Phase of Study Inspected	Date of Inspection	Date of Reporting to Management and Study Director
Protocol:		
draft	April 3, 2009	April 6, 2009
Final	April 6, 2009	April 6, 2009
Amendment 01	April 10, 2009	April 10, 2009
Amendment 02	April 28, 2009	April 28, 2009
Amendment 03	May 7, 2009	May 7, 2009
In-progress study:		
Preparation of feed solutions:	April 10, 2009	April 10, 2009
Introduction of fish:	April 16, 2009	April 16, 2009
Analysis of test fish:	April 20, 2009	April 20, 2009
Analysis of test water:	April 23, 2009	April 23, 2009
Final report:		
draft	June 16, 2009	June 18, 2009
Final	June 26, 2009	June 26, 2009

Quality Assurance Personnel

Yumiko KASHIWAGI, B.Sc.

Sealed date: June 26, 2009

Introduction

1. Study Title

Bioconcentration Study of FRD903 with Carp
(Study No. A080560)

2. Purpose

This study was conducted to evaluate the bioaccumulation potential of the test substance for notification under the Chemical Substances Control Law of Japan.

3. Test Guideline

The study was conducted in accordance with the Test Method Relating to New Chemical Substances <Bioconcentration test of chemical substances in fish and shellfish> (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokiatsu No.031121002, November 21, 2003; the latest revision, November 20, 2006), which prescribes the procedure for testing new chemical substances as required by the Chemical Substances Control Law of Japan.

4. GLP Compliance

The study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) standards concerning the test facility in which the study of new chemical substance is performed (Yakushokuhatsu No.1121003, Heisei 15.11.17 Seikyoku No.3, Kanpokiatsu No.031121004, November 21, 2003; the latest revision, July 4, 2008)

5. Sponsor

DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.
5-18 Sarugaku-chou 1-chome, Chiyoda-ku, Tokyo, Japan

6. Testing Facility (Head Office)

Mitsubishi Chemical Medience Corporation
2-8 Shibaura 4-chome, Minato-ku, Tokyo, Japan

7. Testing Facility (Laboratory)

Yokohama Laboratory, Toxicological Science Division, Medi-Chem Segment,
Mitsubishi Chemical Medience Corporation
1000 Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa, Japan

8. Study Director

Hirokazu KOBAYASHI, M. Sc
Bioaccumulation Evaluation Group

9. Experimental Scientist

Shirou KAWAHARA, M.Sc
(Experimental work)

10. Date

Study initiation:	April 6, 2009
Exposure starting:	April 16, 2009
Exposure completion:	May 14, 2009
Study completion:	June 26, 2009

11. Storage and Retention of Test Substance and Records

The following will be stored in the archives of the Yokohama Laboratory.

- 1) Protocol
- 2) Final report
- 3) Raw data
- 4) Test substance
- 5) Other requirements

Contents

	page
Summary	9
1. Material	11
1.1. Test Substance	11
1.1.1. Identification	11
1.1.2. Source	11
1.1.3. Confirmation of Stability	12
1.2. Test fish	12
1.2.1. Acute Toxicity Test	12
1.2.2. Bioconcentration Test Methods	13
1.3. Dilution Water	13
2. Test Method	14
2.1. Outline of Bioconcentration Test	14
2.1.1. Test Guideline	14
2.1.2. Setting of concentration level	14
2.1.3. Justification for Selection of the Test System	14
2.1.4. Determination of Concentration Level	14
2.1.5. Determination of the Exposure Period	14
2.2. Acute Toxicity Test	14
2.3. Bioconcentration Test Methods	15
2.3.1. Test condition	15
2.3.2. Test system	15
2.3.3. Preparation of test water	16
2.3.4. Introduction of Fish	16
2.3.5. Observations and Environmental Control	16
2.3.6. Analysis of test Substance	18
2.3.7. Validity of the test	18
2.3.8. Calculation of Bioconcentration Factor	18
2.4. Analytical method	19
2.4.1. Apparatus and Reagent	19
2.4.2. High performance liquid chromatograph – Mass spectrometer (LC/MS/MS)	
System and Conditions	20
2.4.3. Calibration Curve	21
2.4.4. Detection Limit	21
2.4.5. Analysis of Test Substance in Test Water	22

2.4.6. Analysis of Test Substance in Test Fish	23
2.4.7. Blank and recovery tests	24
2.4.8. Detection limit of bioconcentration factor	25
2.5. Statistical Methods	25
2.5.1. Correlation of the calibration curve	25
2.5.2. Calculation of mean concentration in test water and BCF	25
3. Results and Discussion	25
3.1. Deviation from protocol	25
3.2. Circumstances That May Have Affected the Reliability of the Test Results	25
3.3. Monitoring of Test Conditions	25
3.4. Concentration of Test Substance in Test Water	26
3.5. BCF of Test Substance in Test Fish	26
3.6. Validity of the test	26
Tables & Figures	27-34
Appendix 1 Information on the test substance	2pp.
Appendix 2 Dilution water quality	4pp.
Appendix 3 Acute toxicity test.....	3pp.
Appendix 4 Calibration curves and chromatograms (Standard solution, blank test and recovery test)	20pp.
Appendix 5 Chromatograms (Analysis of the test substance in the test water)....	19pp.
Appendix 6 Chromatograms (Analysis of the test substance in the test fish)	31pp.

Summary

Sponsor: DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.
Study title: Bioconcentration Study of FRD903 with Carp
Study number: A080560
Study period: April 6, 2009 to June 26, 2009
Methods: The study was conducted in accordance with the Test Method Relating to New Chemical Substances <Bioconcentration test of chemical substances in fish and shellfish> (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokiatsu No.031121002, November 21, 2003; the latest revision, November 20, 2006), which prescribes the procedure for testing new chemical substances as required by the Chemical Substances Control Law of Japan.

Test substance in test water (nominal concentrations)

High concentration level: 0.2 mg/L
 Low concentration level: 0.02 mg/L
 Control: 0 mg/L

Supply of test water: Continuous flow-through dilution system (800 L/day)
 Exposure period: 28 days
 Analytical method: After pretreatment, the test substance was determined by high performance liquid chromatograph-mass spectrometer (LC/MS/MS).
 Lipid content of carp: 3.3% (n=3, 2.0-4.5%) at the start of the exposure period
 3.8% (n=3, 3.5-4.5%) at the end of the exposure period
 96-hour LC₅₀ to Medaka: >100 mg/L

Results: The bioconcentration factors (BCF) obtained in this study were as follows:

Exposure period (days)	4	7	15	21	28
High concentration level					
Mean conc. in test water (mg/L)	0.194	0.199	0.202	0.199	0.198
No. 1	<3	<3	<3	<3	<3
BCF _{SS} <3	No. 2	<3	<3	<3	<3
Low concentration level					
Mean conc. In test water (mg/L)	0.0188	0.0195	0.0193	0.0198	0.0198
No. 1	<30	<29	<29	<28	<28
BCF _{SS} <30	No. 2	<30	<29	<28	<28

BCF_{SS} : The steady-state bioconcentration factor

Discussion

At both concentration levels, the variation of mean BCF at the last three consecutive measurements was not confirmed to fall within $\pm 20\%$. But, because all the BCF values during the exposure period were less than 100, the bioconcentration was considered to

reach the steady-state.

The bioconcentration factors at the steady-state (BCF_{ss}) was <3 at the high concentration level, and <30 at the low concentration level.

Based on the results, the bioaccumulation potential of the test substance in fish tissues is judged to be low.

1. Material

1.1. Test Substance

1.1.1. Identification

Name of the new chemical substance (by IUPAC nomenclature)	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid		
Alternate name	FRD903		
CAS RN	13252-13-6		
Structural formula or rational formula (If both of them are unknown, outline of preparation or manufacturing)	$\text{CF}_3\text{CF}_2\text{CF}_2\text{OCF}(\text{CF}_3)\text{COOH}$		
Molecular weight	330.05		
Purity (%) of the new chemical substance subjected to the study	99.6		
Lot number of the new chemical substance subjected to the study	0711FRD036-2		
Name and content of impurities	Unknown: about 0.4%		
Vapor pressure	About 1 mmHg (25°C)		
Solubility in water	20 wt%		
1-octanol/water partition coefficient	—		
Melting point	—		
Boiling point	About 85°C /38 mmHg		
Appearance at room temperature	Colorless and clear liquid		
Stability	—		
Solubility in solvents and the like	Solvent	Solubility	Stability in solvents
	Acetone	Above 25 wt%	—

Above information is provided by the sponsor.

1.1.2. Source

Supplier : DU PONT-MITSUI FLUOROCHEMICALS COMPANY, LTD.

1.1.3. Confirmation of Stability

During the study, the test substance was sealed and stored in a desiccator (stored condition: room temperature and dark). At the end of the study, the infrared absorption spectrum of the test substance was measured. The spectrum was consistent with that measured at the start of the study, indicating that the test substance was stable under the storage condition.

[Appendix 1]

Apparatus: Infrared spectrophotometer,
Nicolet iS10, Thermo Fisher Scientific Inc.
AVATAR 320, Nicolet Instrument Corporation

1.2. Test Fish

1.2.1 Acute Toxicity Test

Species: Medaka (*Oryzias latipes*)
Source: Tsunashima Fishing Co., 5-18-1 Tsunashimanishi, Kouhoku-ku,
Yokohama, Kanagawa, Japan
Date of purchase: January 29, 2009
Lot number: 09-H-0129
Pre-acclimation: At the start of acclimation, visual observation was made and abnormal fish was removed. The remaining normal fish were reared in an aquarium with flowing water.
Body length: 2 ± 1 cm
Body weight: Approximately 0.2 g
Acclimation:
Aquarium number: C-2
Water temperature: $24 \pm 1^\circ\text{C}$
Period: March 25, 2009 to April 7, 2009
Feeding: The food (Tetramin[®], Tetra Werke) was given in an amount of 2% of the fish weight everyday. (Feeding was discontinued during the 24 hours before testing.)
Mortality during the 1 week before testing: <5%
Medication during acclimation: None

1.2.2 Bioconcentration Test Methods

Species:	Carp (<i>Cyprinus carpio</i>)
Source:	Niikura Fish Farm 1217, Shimoya, Isehara-city, Kanagawa, Japan
Date of purchase:	July 22, 2008
Lot number:	08-K-0722
Pre-acclimation:	At the start of acclimation, visual observation was made and abnormal fish were removed. The remaining normal fish were then reared in an aquarium with flowing water.
Total length:	8 ± 4 cm
Body weight:	Approximately 5 g
Age:	Less than a year after hatching

2) Acclimation

Aquarium number:	A-15
Water temperature:	24 ± 2 °C
Period:	March 24, 2009 to April 16, 2009
Food:	Babygold®, Kyorin
Feeding:	The food was given in an amount of 2% of the fish weight everyday.
Mortality during the 1 week before testing:	<5%
Medication during acclimation:	None

1.3. Dilution Water

To prepare the dilution water, Yokohama municipal tap water was treated with activated charcoal, and sodium thiosulfate solution was added to neutralize trace free chlorine. This dilution water quality is checked half-yearly by Mitsubishi Chemical Analytech Co., Ltd.

[Appendix 2]

2. Test Method

2.1. Outline of Bioconcentration Test

2.1.1. Test Guideline

The study was conducted according to the Test Method Relating to New Chemical Substances <Bioconcentration test of chemical substances in fish and shellfish> (Yakushokuhatsu No.1121002, Heisei 15.11.13 Seikyoku No.2, Kanpokiatsu No.031121002, November 21, 2003; the latest revision, November 20,2006), which prescribes the procedure for testing new chemical substances as required by the Chemical Substances Control Law of Japan.

2.1.2. Setting of concentration level

The bioconcentration test system was set up in duplicate for different concentrations and two groups of carp was kept in water containing the test substance. In addition, the system was also set up for control and a group of carp was kept in water without the test substance. During the exposure period, the concentrations of the test substance in water and fish were measured on predetermined days. The bioconcentration factors (BCF) calculated from the values was used to evaluate bioaccumulation potential of the test substance in tissues of fish.

2.1.3. Justification for Selection of the Test System

Carp was selected for test fish since it is one of the recommended species in the test guideline. The criteria in selection of species were that they could be satisfactorily maintained in the laboratory, many fish of one batch was available in convenient size and in good health condition.

2.1.4. Determination of the Concentration Level

From the result of the preliminary acute toxicity test with Medaka, the 50% lethal concentration (LC_{50}) for 96 hours of the test substance was >100 mg/L. Therefore the bioconcentration test was accordingly conducted at exposure concentration levels of 0.2 and 0.02 mg/L, less than a hundredth and a thousandth of the 96-hour LC_{50} .

2.1.5. Determination of the Exposure Period

The exposure period was set to be 28 days, because the bioaccumulation possibility of the test substance in fish was presumed low under the prescribed test condition.

2.2. Acute Toxicity Test

The acute toxicity test was performed, and 96-hour LC_{50} to Medaka were calculated. The 96-hour LC_{50} of the test substance was >100 mg/L, and the nominal concentration of bioconcentration test was confirmed to be less than a hundredth and a thousandth of the 96-hour LC_{50} .

[Appendix 3]

2.3. Bioconcentration Test Method

2.3.1. Test condition

- 1) Supply of test water: Continuous flow-through dilution system
- 2) Exposure level (nominal concentrations of the test substance)
 - High concentration level: 0.2 mg/L
 - Low concentration level: 0.02 mg/L
 - Control: 0 mg/L
- 3) Supply of dilution water: 800 L/day (Turnover rate: 16 times/day)
- 4) Supply of feed solution: 20 mL/day
- 5) Number of fish (at the start of the exposure period):
 - High concentration level: 28 fish in 50-L test water (>1 L/day/g)
 - Low concentration level: 28 fish in 50-L test water (>1 L/day/g)
 - Control: 12 fish in 50-L test water (>1 L/day/g)
- 6) Water temperature: $24 \pm 2^\circ\text{C}$
- 7) Dissolved oxygen: $\geq 60\%$ of the saturation ($\geq 5\text{mg/L}$ at 24°C)
- 8) Aeration: Continuous
- 9) pH: 6.0 – 8.5
- 10) Lighting: Approximately 16 hr/day
(Hf fluorescent lamp with wavelengths of 400-700 nm)
- 11) Exposure Period: 28 days

2.3.2. Test system

The outline was shown in Figure 1 (p.27).

2.3.3. Preparation of test water

1) Preparation of Feed Solutions

High concentration level

Test substance, 2 g
 |
 ←Purified water
Dissolve and dilute to 250 mL (Feed solution 1)
 Concentration of test substance: 8000 mg/L

Low concentration level

Feed solution 25 mL
 |
 ←Purified water
Dissolve and dilute to 250 mL (Feed solution 2)
 Concentration of test substance: 800 mg/L

Feed solutions were renewed within 14 days.

2) Preparation of Test Solutions

Each feed solution prepared in 1) was supplied to a mixing glass tube with a metering pump for the feed solution, diluted to the respective nominal concentration by mixing with dilution water delivered through a metering pump for dilution water, and then supplied to each test chamber.

[Figure 1 (p. 27)]

2.3.4. Introduction of Fish

The bioconcentration test system was operated with no fish until HPLC analysis proves that the test substance concentrations in both test chambers were kept at respective nominal concentrations. Fish was introduced into the test chamber and the bioconcentration test was started.

2.3.5. Observations and Environmental Control

1) Conditions of fish

Observations were made on the appearance, swimming and eating behavior of fish, and the results were recorded every day.

2) Feeding

Babygold® was given in an amount of 2 % of fish weight everyday.

3) Measurement of dissolved oxygen and temperature

The water quality parameters were measured at water sampling day during exposure period.

Apparatus: DO meter, DOL-10, DKK Corporation
Digital Thermometer, TX1001, Yokogawa Meters & Instruments Corp.

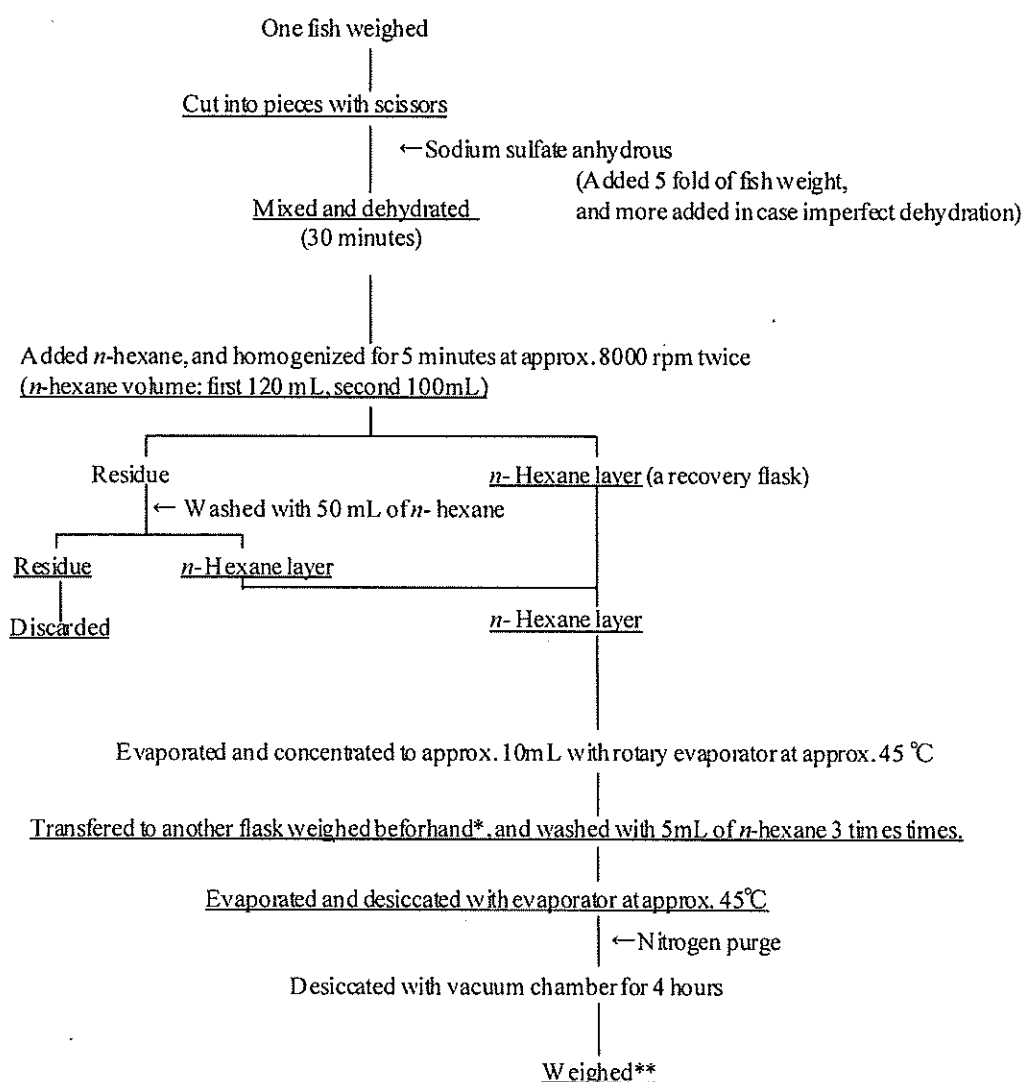
4) Measurement of pH

At the start and the end of the exposure period, pH of the test water was measured.

Apparatus: pH meter; Toa Electronics, Model HM-40V

5) Measurement of lipid content

At the start and the end of the exposure, lipid content of the test fish was measured. At the start of the exposure period, five fish was taken from acclimation aquarium. At the end of the exposure period, another five fish was also taken from control aquarium. Lipid contents of three of them (n=3) was measured respectively. The remaining fish slurry at the pre-treatment for fish analysis will be stored for 3 months in a freezer in case of additional measurement, or they may be discarded.



Equations : lipid weight (g) = lipid and flask weight (**) - flask weight (*)

lipid content (%) = lipid weight (g) / fish wet weight (g) x 100

2.3.6. Analysis of test substance

During the exposure period, concentration of the test substance in test water and test fish were analyzed.

1) The test substance in test water

On days 0, 4, 7, 15, 21 and 28 of the exposure period, test water was analyzed as described in 2.4.5.

2) The test substance in test fish

On days 4, 7, 15, 21 and 28 of the exposure period, four fish was taken from each test chamber and four of them was analyzed two by two by the method described in 2.4.6.

The residual fish will be stored for 3 months in a freezer in case of additional measurement, or they may be discarded.

2.3.7. Validity of the test

Validity of the test was decided from the following conditions.

- The temperature variation is less than ± 2 °C.
- The concentration of dissolved oxygen does not fall below 60% saturation.
- The concentration of the test substance in the chambers is maintained within $\pm 20\%$ of the mean of the measured values during the exposure period. (There are some cases where this rule does not apply if the bioconcentration factor of the test substance in the fish tissues is very high. In such cases, the range of the concentration in the test water should be maintained within $\pm 20\%$ of the mean value at steady state.)
- The mortalities or abnormalities in both control and treated fish are less than 10% at the end of the test. In case the test is extended over several weeks or months, mortalities or abnormalities in both sets of fish are less than 5 % per month and not exceed 30 % in all.

2.3.8. Calculation of Bioconcentration Factor

The bioconcentration factor (BCF) is calculated by the following equation:

$$BCF_n = C_{f,n} / C_{w,n}$$

The steady-state bioconcentration factor (BCF_{ss}) is calculated by the following equation:

$$BCF_{ss} = C_{f,s} / C_{w,s}$$

Where BCF_n : The bioconcentration factor after n days

BCF_{ss} : The steady-state bioconcentration factor

$C_{f,n}$: Concentration of the test substance in test fish after n days ($\mu\text{g/g}$)

$C_{w,n}$: Mean concentration of the test substance in test water over n days (mg/L)

$C_{f,s}$: Mean concentration in test fish at steady-state ($\mu\text{g/g}$)

$C_{w,s}$: Mean concentration in test water at steady-state (mg/L)

Steady-state: Steady-state is decided to be reached, when three successive BCF at intervals of 48 hours or longer are within 20% of each other. $C_{f,s}$ and $C_{w,s}$ are calculated from last three successive data.

When the obtained BCF are all less than 100, it is also considered that steady-state is reached. In this case, three successive BCF are not

necessary within 20% of each other. The BCFss is not calculated by above equation and BCFss is just declared "< (maximum BCF value) "or \leq (maximum BCF value).

2.4. Analytical method

2.4.1. Apparatus and Reagent

1) Apparatus

Electrobalance:	AG204, Mettler K.K. PB3002, Mettler K.K. PB3002-S, Mettler-Toledo K.K.
Homogenizer:	AM, Nihon Seiki Kaisha Ltd.
Aspirator:	WP-15, Yamato Scientific Co. Ltd. PP-11, Masuda Corporation
Rotary evaporator:	RE-111, Sibata Scientific Technology Ltd. RE-400, Yamato Scientific Co. Ltd.
Cooling aspirator:	CF600P, Yamato Scientific Co. Ltd.

2) Reagents

Methanol:	HPLC reagent, Wako Pure Chemical Industries., Ltd. Guaranteed reagent, Junsei Chemical Co., Ltd.
Formic acid:	Guaranteed reagent, Wako Pure Chemical Industries, Ltd.
Ammonium formate:	Wako reagent, Wako Pure Chemical Industries, Ltd.
Purified water:	Grade A4 in Japanese Industrial Standards (JIS) K0557
n-Hexane:	Guaranteed reagent, Junsei Chemical Co., Ltd.
Anhydrous sodium sulfate:	Guaranteed reagent, Junsei Chemical Co., Ltd.

2.4.2. High performance liquid chromatograph – Mass spectrometer (LC/MS/MS) System and Conditions

System

High performance liquid chromatograph mass spectrometer (LC/MS/MS)
 : UPLC Q-Premier XE (No.1)
 Workstation : MassLynx 4.1
 High performance liquid chromatograph (HPLC) : ACQUITY UPLC
 Pump : Binary Solvent Manager
 Auto injector : Sample Manager
 Mass spectrometric detector (MSD) : Quattro Premier XE

Conditions

(HPLC conditions)

Column : Waters Co.,
 VanGuard™ Pre-column ACQUITY UPLC BEH C18, 1.7 µm, 2.1 mm
 i.d.× 5 mm + ACQUITY UPLC BEH C18, 1.7 µm, 1.0 mm i.d.× 150 mm
 Oven temperature : 60°C
 Mobile phase : A1 : 20 mM ammonium formate aqueous solution : formic acid =
 1000 : 1
 B1 : methanol (HPLC grade)

Gradient conditions

Time	A1	B1
0.00 min	45%	55%
2.50 min	45%	55%
3.00 min	10%	90%
3.50 min	45%	55% (step gradient)

Measurement time : 4.0 min
 Flow rate : 0.12 mL/min
 Injection volume : 5 µL
 Injection mode : Partial Loop With Needle Overfill

(MSD conditions)

Ion mode : Negative (Electrospray)
 Detection mode : MRM
 Monitoring ion : *m/z* Parent 328.87, Daughter 284.80
 Voltage : Capillary 1.00 kV
 Cone 10 V
 Collision energy 14.0 eV
 Temperatures : Source Temp 100 °C
 Desolvation Temp 300 °C
 Gas Flow : Desolvation 500 L/hr
 Cone 50 L/hr
 Collision Gas Flow 0.10 mL/min

2.4.3. Calibration Curve

The test substance was dissolved in purified water at the concentration of 1000 mg/L. Then it was diluted sequentially with purified water to prepare standard solutions of 0.01, 0.02 and 0.04 mg/L. Purified water was used as 0 mg/L standard solution. These standard solutions were analyzed with LC/MS/MS. The peak area (count) for the test substance was plotted against its concentration. Each calibration curve drawn by the least square method was straight line almost passing through the origin and gave a satisfactory correlation-coefficient of 0.9998.

[Appendix 4]

2.4.4. Detection limit

In the LC/MS/MS analysis, detection limit was set at 0.001 mg/L, which corresponds to 5% of the standard solution (0.02 mg/L).

2.4.5. Analysis of Test Substance in Test Water

The test substance in test water was analyzed according to the following procedures.

Prior to each analysis, standard solution of 0.02 mg/L was analyzed. The test substance concentration was determined from the ratio of peak area of the sample to that of the standard solution. In the following 2.4.6 and 2.4.7., the test substance concentration was determined in a same manner.

High concentration level

Test water sample

Dilute with purified water (Dilution ration: 10)

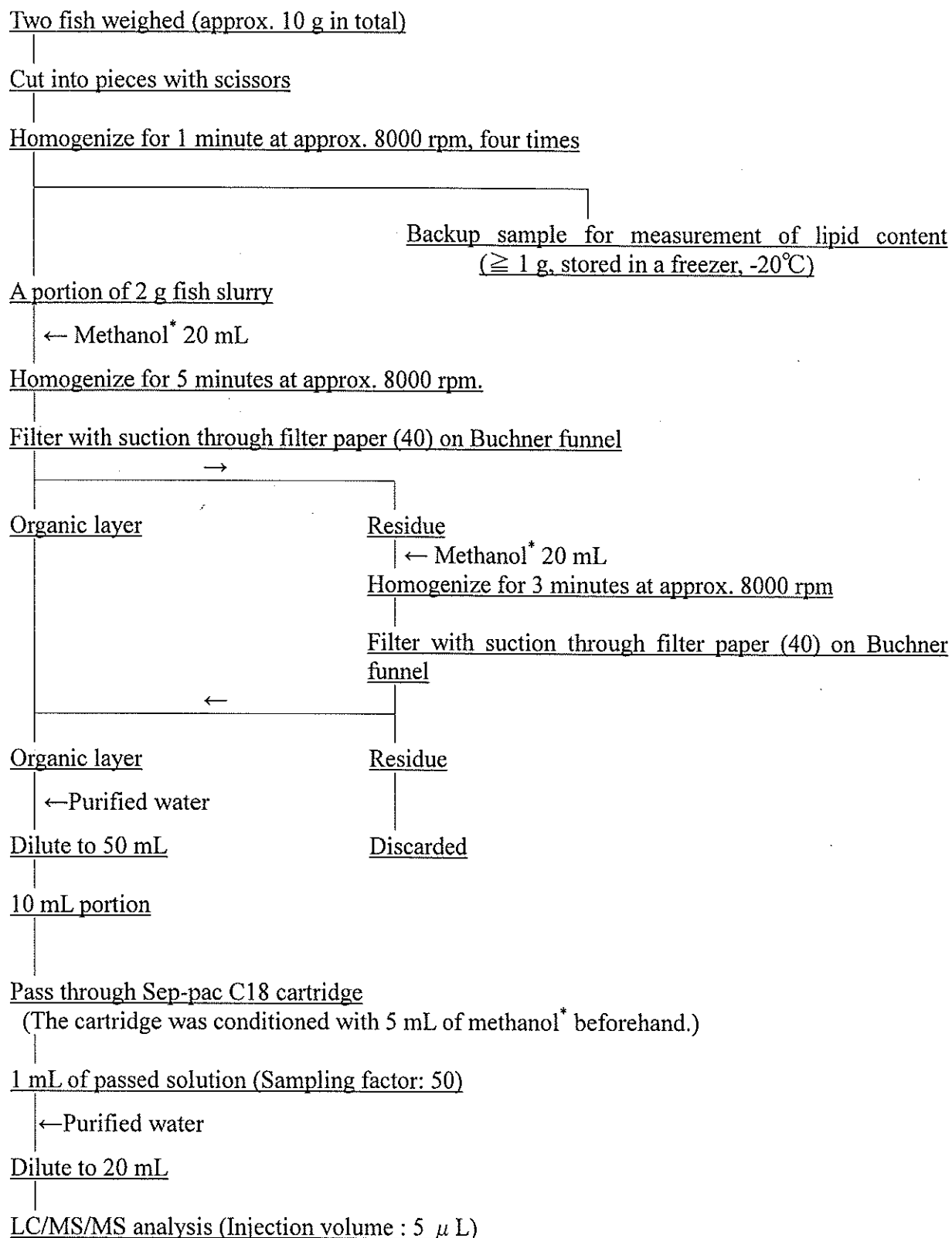
LC/MS/MS analysis

Low concentration level

Test water sample

LC/MS/MS analysis

2.4.6. Analysis of Test Substance in Test Fish



Sep-pac C18 cartridge : Waters Sep-pac plus C18 cartridges
 *Methanol: Guaranteed reagent

2.4.7. Blank and recovery tests

1) Test water

Blank test

Prior to the exposure period, blank test was performed by using the water in the acclimatization tank. The water sample was analyzed twice according to the method of the low concentration level described in 2.4.5. The background values was confirmed to be smaller than the detection limit, and the concentration of the test substance in the water was calculated to be <0.001 mg/L.

[Table 1 (p.28), Appendix 4]

Recovery test

No recovery test was performed because dilution operation alone was performed in the analysis of the test waters for low concentration levels and no operation was performed in analysis of the test waters for high concentration level as shown in 2.4.5.

2) Test fish

Blank test

Prior to the exposure period, blank test was performed with four fish sampled from the acclimatization tank. And at the end of the exposure period, another four fish was also sampled from the control group. Fish was analyzed four times according to the method described in 2.4.6. The background values was confirmed to be smaller than the detection limit, and the concentration in fish was calculated to be <0.50 $\mu\text{g/g}$.

[Table 2 (p.29), Appendix 4]

Recovery test

Prior to exposure period, eight fish was sampled from the acclimatization tank and the recovery test was conducted. Zero point two-milliliter of the 100 mg/L methanol (Guaranteed reagent) solution of the test substance was added to 2 g of fish slurry which sampled according to the method described in 2.4.6. The concentration of the test substance in the fish sample is 10 $\mu\text{g/g}$, which is 50-fold of the high concentration level (0.2 mg/L). Four measurements of recovery gave the mean value of 90.2%, and the standard deviation of 1.6%, with differences between maximum and minimum of 3.4%, which were satisfactory for quantitative analysis. The average recoveries were used to correct the measured values in fish.

[Table 3 (p.30), Appendix 4]

2.4.8. Determination limit of bioconcentration factor

The blank test for the test fish showed that the concentration of the test substance in the fish was lower than the detection limit of $<0.50 \mu\text{g/g}$. This value was 2.5 and 25 times the nominal concentration of the test water for the high (0.2 mg/L) and low (0.02 mg/L) concentration level, respectively. In other words, the determination lower limits of the bioconcentration factors were about 3 and 30 for the high and low concentration level. However, the actual lower limit might depend on deviations of the test substance concentration in the test water and recovery rate in the fish analysis.

2.5. Statistical Methods

2.5.1. Correlation of the calibration curve

The correlation-coefficient was calculated by least square method according to JIS Handbook "General rules for chemical analysis".

- JIS K0050, General rules for chemical analysis

2.5.2. Calculations of mean concentration in test water and BCF

The mean concentration in test water and BCF was calculated by the method according to JIS Handbook "Guide to the handling of numbers".

- JIS Z9041-1:1999, Statistical interpretation of data – Part 1: Statistical presentation of data
- JIS Z8401-1:1999, Guide to the rounding of numbers

3. Results and Discussion

3.1. Deviation from protocol

It was settled in protocol that observations and feeding of the test fish would be made every day. However, no observation and feeding were made at days 20 of the exposure period (May, 6, 2009).

This case was judged not to affect to the test results for 2 reasons described as follows:

- No abnormality was observed for the test fish at next day
- It was considered that no feeding did not affect to bioconcentration factor because lipid content value of the test fish was stayed flat at the start and the end of exposure period.

3.2. Circumstances That May Have Affected the Reliability of the Test Results

There was no significant matter that may have affected the reliability of the test results.

3.3. Monitoring of Test Conditions

During the test period, the dissolved oxygen in all water chambers was kept at $\geq 60 \%$ (7.2-8.4 mg/L) of the saturated oxygen concentration and the water temperature was kept at $24^\circ\text{C} \pm 2^\circ\text{C}$ (24.0-24.6°C), both of which satisfied the test conditions. The pH value was kept at 7.4-8.0, which was within the proper range for fish rearing (6.0-8.5). During the test period,

there was no abnormality in shape of the body or in swimming and eating behavior in the fish at all the concentration levels throughout the test period. Therefore, it was confirmed that the test fish had been reared appropriately.

Lipid content: 3.3% (n=3, 2.0-4.5%) at the start of the exposure period

3.8% (n=3, 3.5-4.5%) at the end of the exposure period

The average at the end of the exposure period fell within $\pm 25\%$ range of the average at the start of the exposure period.

3.4. Concentration of Test Substance in Test Water

The mean concentration of the test substance during the exposure period was 0.198 mg/L at the high concentration level, and 0.0198 mg/L at the low concentration level and sufficiently close to the nominal values. The coefficients of variation for concentration were 4.5% and 6.1% at the high and low concentration level. The variation of the test substance concentrations in the test waters at both concentration levels were kept within $\pm 20\%$ of the mean measured values.

[Table 4-5 (p.31-32), Figure 3-4 (p.31-32), Appendix 5]

3.5. BCF of Test Substance in Test Fish

The bioconcentration factor (BCF) during the exposure period was <3 at the high concentration level, and <28 to <30 at the low concentration level.

At both concentration levels, the variation of mean BCF at the last three consecutive measurements was not confirmed to fall within $\pm 20\%$. But, because all the BCF values during the exposure period were less than 100, the bioconcentration was considered to reach the steady-state.

The bioconcentration factors at the steady-state (BCF_{ss}) was <3 at the high concentration level, and <30 at the low concentration level.

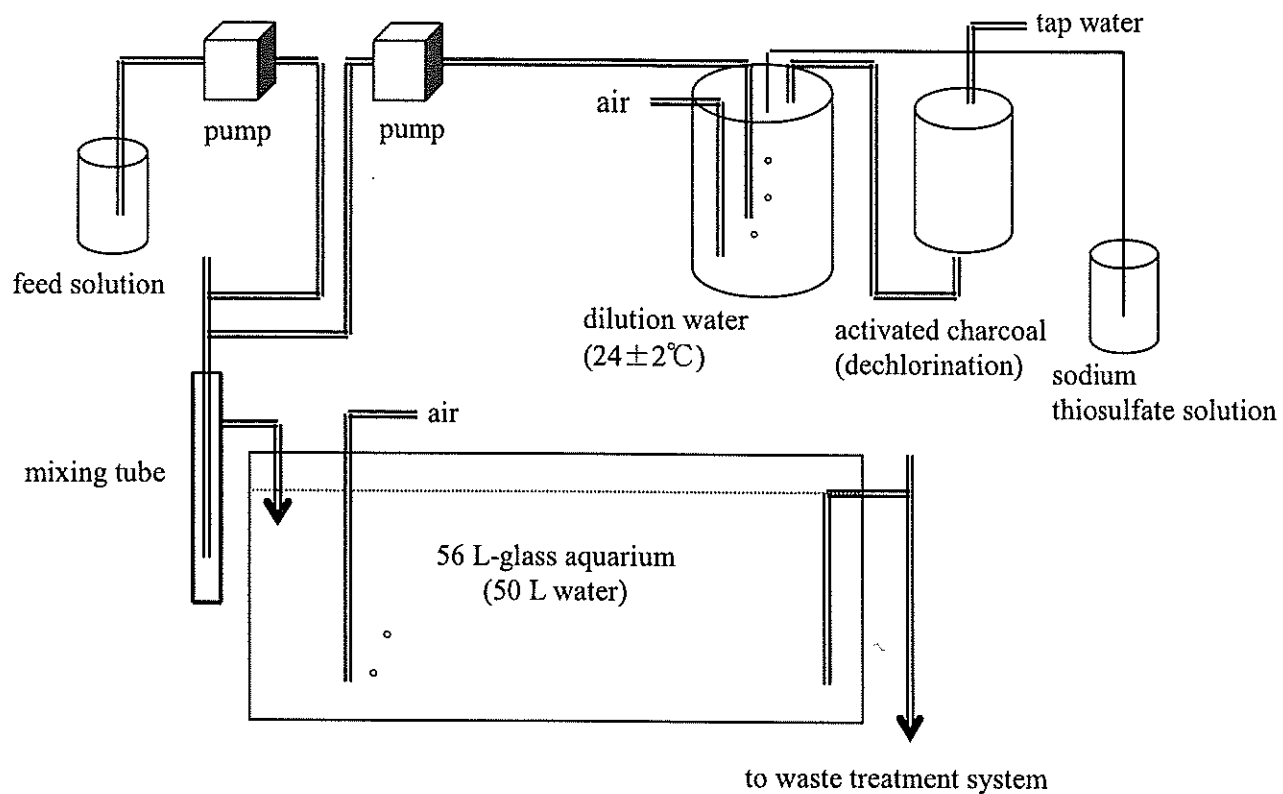
Based on the results, the bioaccumulation potential of the test substance in fish tissues is judged to be low.

[Table 6-7 (p. 33-34), Appendix 6]

3.6. Validity of the Test

This test is judged valid because all results satisfied the validity criteria described in §2.3.7.

Figure 1 Bioconcentration test system



(Apparatus)

Pump (dilution water supply)

Exposure: FMI Co., QD-2CSC

Control: FMI Co., QD-2CSC

Pump (feed solution supply)

Exposure: Nihon Seimitsu Kagaku Co., NRX-01-L

Table 1 Blank test of the test substance in water

	A	B	C
analysis	peak area count		conc. in water mg/L
No.	sample	std.	
1	ND	1675.8	<0.001
2	ND	1675.8	<0.001

Concentration of std. solution (Cstd) :

0.02 mg/L

Detection limit to concentration of std. solution (DL) :

5 %

ND : Not detected

Equation : $C = (B \times (DL/100)) \div B \times Cstd$

Table 2 Blank test of the test substance in fish

		W	A	B	C	D
analysis	sampling	fish weight	peak area count		conc. in final solution	conc. in fish
No.	No.	g	sample	std.	mg/L	µg/g
1	J-1	5.48	ND	1371.5	<0.0010	<0.50
	J-2	6.71				
2	J-3	5.36	ND	1371.5	<0.0010	<0.50
	J-4	5.82				
3	C-1	5.68	ND	670.9	<0.0010	<0.50
	C-2	7.23				
4	C-3	7.52	ND	670.9	<0.0010	<0.50
	C-4	7.54				

Concentration of std. solution (Cstd) : 0.02 mg/L
 Final volume (FV) : 20 mL
 Sampling factor (SF) : 50
 Detection limit to concentration of std. solution (DL) : 5 %
 Sampling weight of tissue(SW) : 2 g

ND : Not detected

Equations : $C = (B \times (DL/100)) \div B \times Cstd$
 $D = (B \times (DL/100)) \div B \times Cstd \times (FV/1000) \times SF \div SW \times 1000$

Table 3 Recovery test of the test substance in fish

			A	B	C	D	E	R
analysis	sampling	fish weight	test substance added	peak area count		conc. in final solution	measured amount	recovery
No.	No.	g	mg	sample	std.	mg/L	mg	%
1	J-5	5.93	0.02	1291.5	1400.0	0.0185	0.0185	92.3
	J-6	6.13						
2	J-7	4.21	0.02	1245.4	1400.0	0.0178	0.0178	89.0
	J-8	5.43						
3	J-9	4.34	0.02	1270.0	1400.0	0.0181	0.0181	90.7
	J-10	5.38						
4	J-11	4.03	0.02	1245.0	1400.0	0.0178	0.0178	88.9
	J-12	6.96						
Average, n=4 :								90.2
Standard deviation :								1.6
Range :								3.4

Concentration of std. solution (Cstd) : 0.02 mg/L

Final volume (FV) : 20 mL

Sampling factor (SF) : 50

Equations :

$$D = B \div C \times Cstd$$

$$E = B \div C \times Cstd \times (FV/1000) \times SF$$

$$R = B \div C \times Cstd \times (FV/1000) \times SF \div A \times 100$$

Table 4 Concentration of the test substance in the test water - High concentration level

		i	A	B	C	D
sampling date	period	number of analysis	peak area count		conc. in water	mean conc. in water
	day		sample	std.	mg/L	mg/L
2009/04/16	0	1	1086.4	1131.8	0.192	0.192
2009/04/20	4	2	1128.7	1156.0	0.195	0.194
2009/04/23	7	3	1025.3	983.2	0.209	0.199
2009/05/01	15	4	824.4	784.3	0.210	0.202
2009/05/07	21	5	876.7	938.4	0.187	0.199
2009/05/14	28	6	603.9	623.4	0.194	0.198

Standard deviation : 0.009 mg/L

Coefficient of variation : 4.5 %

Concentration of std. solution (Cstd) : 0.02 mg/L

Dilution ratio (DR) : 10

Equations : $C = A \div B \times Cstd \times DR$
 $D = \Sigma(A \div B \times Cstd \times DR) \div i$

Figure 3 Concentration of the test substance in the test water - High concentration level

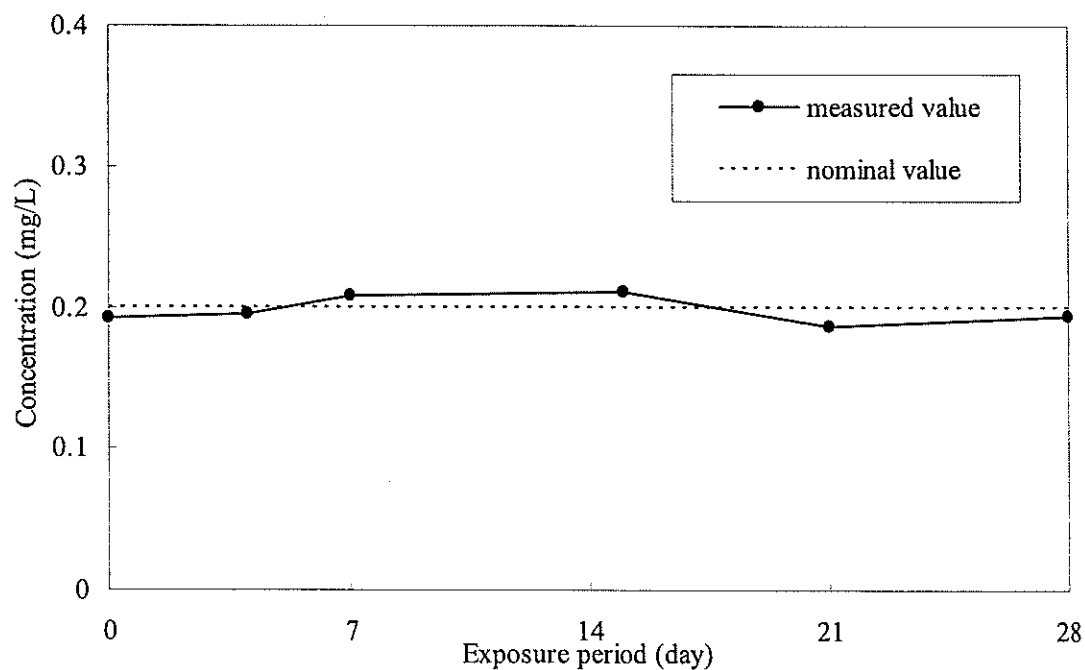


Table 5 Concentration of the test substance in the test water - Low concentration level

sampling date	period day	i	A	B	C	D
		number of analysis	peak area count sample	std.	conc. in water mg/L	mean conc. in water mg/L
2009/04/16	0	1	1057.0	1131.8	0.0187	0.0187
2009/04/20	4	2	1093.5	1156.0	0.0189	0.0188
2009/04/23	7	3	1020.9	983.2	0.0208	0.0195
2009/05/01	15	4	746.4	784.3	0.0190	0.0193
2009/05/07	21	5	1014.0	938.4	0.0216	0.0198
2009/05/14	28	6	620.4	623.4	0.0199	0.0198

Standard deviation : 0.0012 mg/L

Coefficient of variation : 6.1 %

Concentration of std. solution (Cstd) : 0.02 mg/L

Equations : $C = A + B \times C_{std}$
 $D = \sum (A + B \times C_{std}) / i$

Figure 4 Concentration of the test substance in the test water - Low concentration level

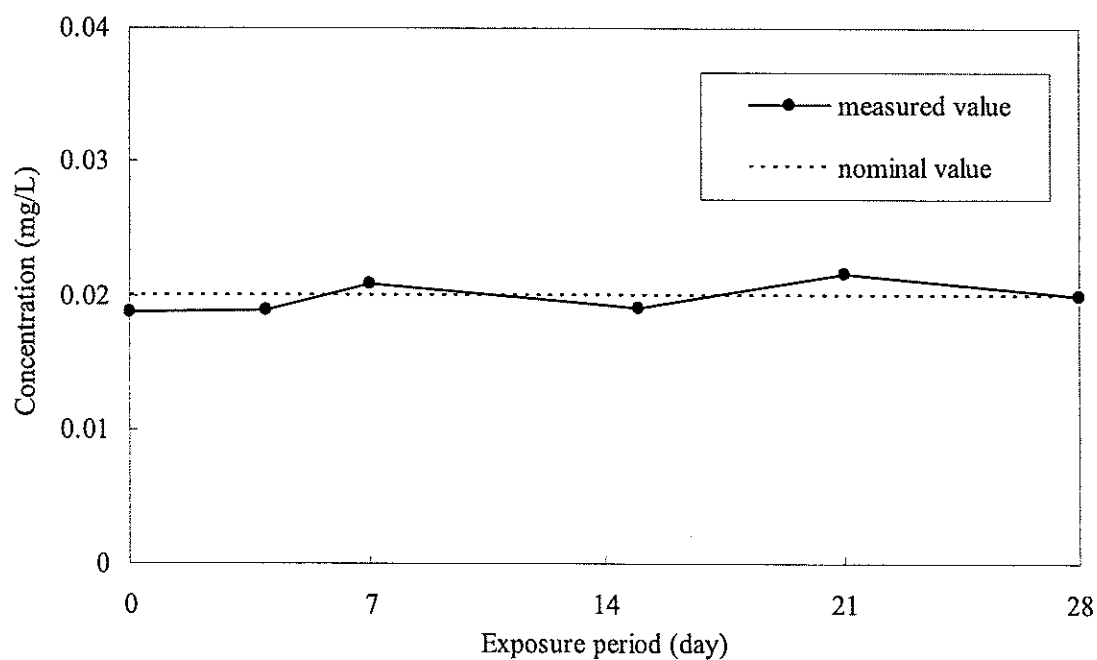


Table 6 Concentration of the test substance in the test fish and bioconcentration factor (BCF)
- High concentration level

exposure period	analysis No.	W1	W2	A	B	C	D	E	F	G
		fish weight		peak area		conc. in final solution	conc. in fish body	mean conc. in water	BCF	mean BCF
		No.1 g	No.2 g	count sample	std. std.	mg/L	µg/g	mg/L		
4	1	3.96	6.10	ND	1047.4	<0.0010	<0.55	0.194	<3	NA
4	2	3.95	5.79	ND	1047.4	<0.0010	<0.55	0.194	<3	
7	1	4.02	6.63	ND	964.1	<0.0010	<0.55	0.199	<3	NA
7	2	6.34	4.70	ND	964.1	<0.0010	<0.55	0.199	<3	
15	1	6.31	4.50	ND	816.1	<0.0010	<0.55	0.202	<3	NA
15	2	5.38	6.60	ND	816.1	<0.0010	<0.55	0.202	<3	
21	1	7.93	7.44	ND	736.8	<0.0010	<0.55	0.199	<3	NA
21	2	7.56	8.92	ND	736.8	<0.0010	<0.55	0.199	<3	
28	1	8.17	5.44	ND	612.0	<0.0010	<0.55	0.198	<3	NA
28	2	7.46	6.81	ND	612.0	<0.0010	<0.55	0.198	<3	

Concentration of std. solution (Cstd) : 0.02 mg/L
 Final volume (FV) : 20 mL
 Sampling factor (SF) : 50
 Recovery (R) : 90.2 %
 Detection limit to concentration of std. solution (DL) : 5 %
 Sampling weight of tissue (SW) : 2 g

ND: Not Detected
 NA: Not Available

Equations :

$$C = (B \times (DL/100)) \div B \times Cstd$$

$$D = (B \times (DL/100)) \div B \times Cstd \times (FV/1000) \times SF \div SW \times 1000 \div (R/100)$$

$$F = (B \times (DL/100)) \div B \times Cstd \times (FV/1000) \times SF \div SW \times 1000 \div (R/100) \div E$$

$$G = (F1 + F2) \div 2$$

Table 7 Concentration of the test substance in the test fish and bioconcentration factor (BCF)
- Low concentration level

exposure period	analysis No.	W1	W2	A	B	C	D	E	F	G
		fish weight		peak area		conc. in final	conc. in fish	mean conc.	BCF	mean BCF
		No.1 g	No.2 g	count sample	std. std.	solution mg/L	body µg/g	in water mg/L		
4	1	5.02	5.51	ND	974.7	<0.0010	<0.55	0.0188	<30	NA
4	2	4.16	6.54	ND	974.7	<0.0010	<0.55	0.0188	<30	
7	1	4.03	5.22	ND	1009.7	<0.0010	<0.55	0.0195	<29	NA
7	2	4.20	4.16	ND	1009.7	<0.0010	<0.55	0.0195	<29	
15	1	6.81	6.84	ND	843.6	<0.0010	<0.55	0.0193	<29	NA
15	2	4.79	8.10	ND	843.6	<0.0010	<0.55	0.0193	<29	
21	1	5.11	7.35	ND	715.8	<0.0010	<0.55	0.0198	<28	NA
21	2	6.70	9.22	ND	715.8	<0.0010	<0.55	0.0198	<28	
28	1	9.77	6.45	ND	608.2	<0.0010	<0.55	0.0198	<28	NA
28	2	8.07	7.53	ND	608.2	<0.0010	<0.55	0.0198	<28	

Concentration of std. solution (Cstd) : 0.02 mg/L
 Final volume (FV) : 20 mL
 Sampling factor (SF) : 50
 Recovery (R) : 90.2 %
 Detection limit to concentration of std. solution (DL) : 5 %
 Sampling weight of tissue (SW) : 2 g

ND: Not Detected
 NA: Not Available

Equations :

$$C = (B \times (DL/100)) \div B \times Cstd$$

$$D = (B \times (DL/100)) \div B \times Cstd \times (FV/1000) \times SF \div SW \times 1000 \div (R/100)$$

$$F = (B \times (DL/100)) \div B \times Cstd \times (FV/1000) \times SF \div SW \times 1000 \div (R/100) \div E$$

$$G = (F1 + F2) \div 2$$

Appendix 1

Information on the test substance

(2 pages)

Figure 1 Infrared absorption spectrum of the test substance before the start of the study

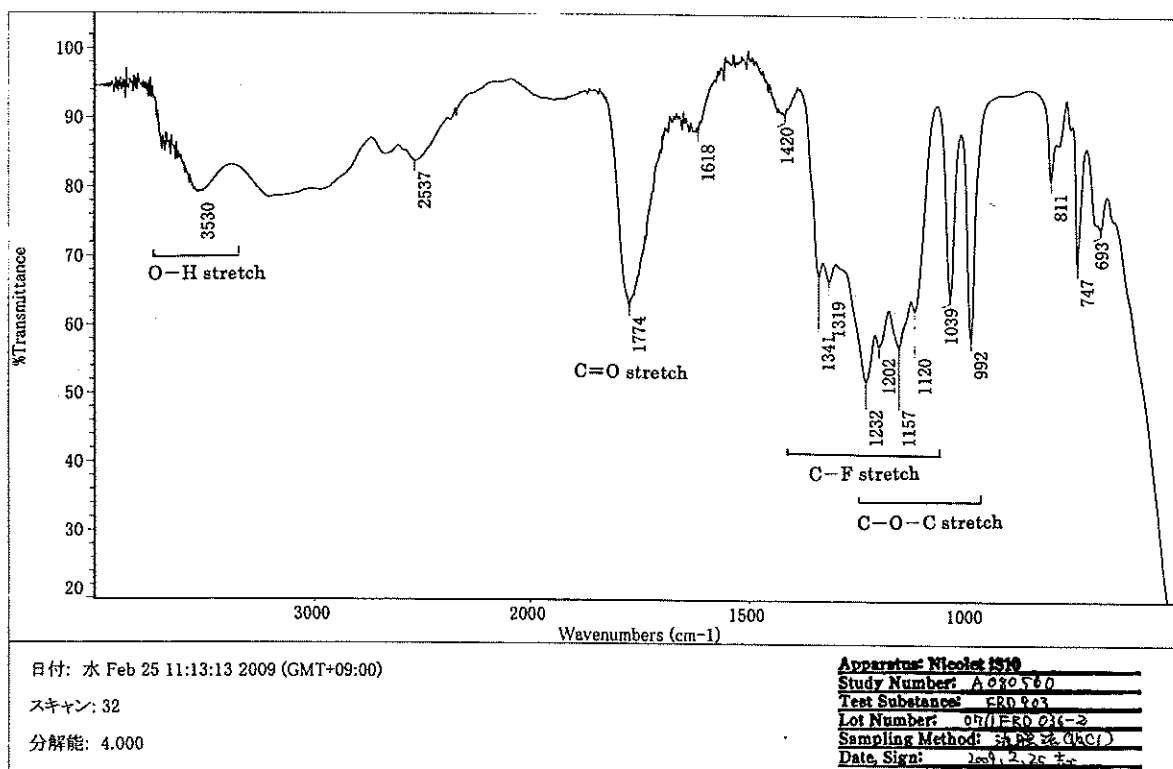
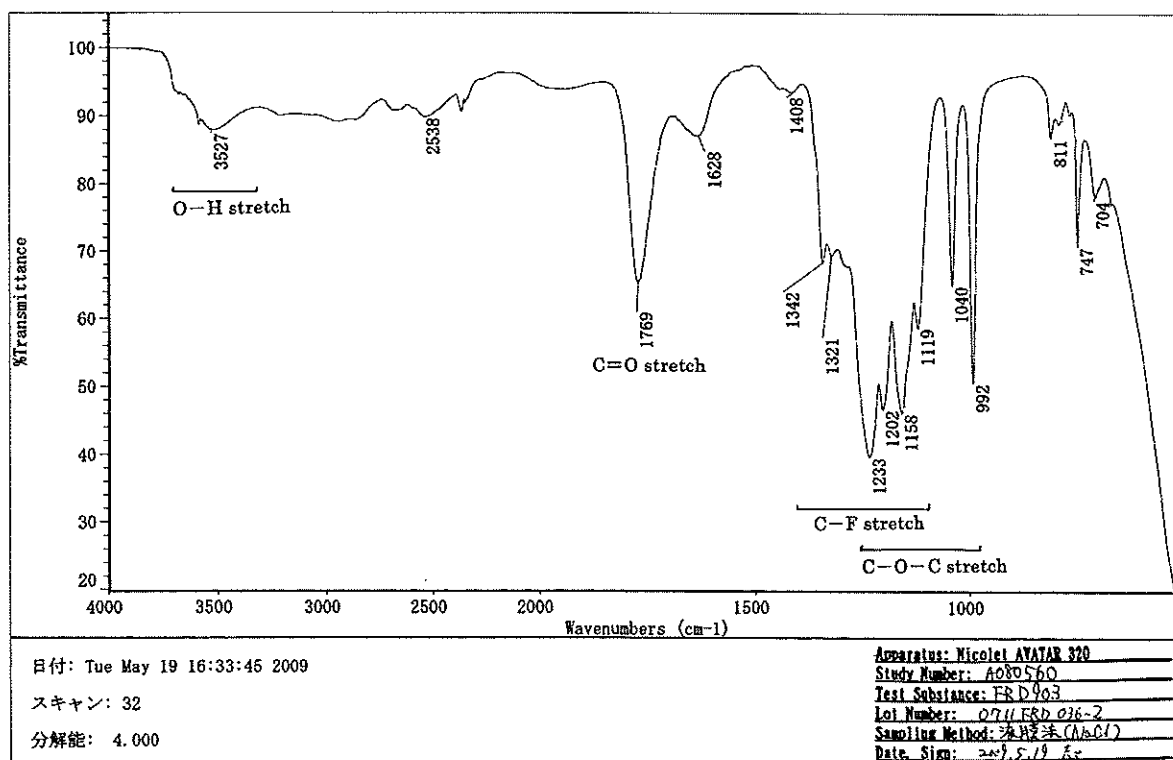


Figure 2 Infrared absorption spectrum of the test substance after the end of the study



Appendix 2

Dilution water quality

(4 pages)

(報告様式-C3C) [1/3]
第08E14729号[2]

測定分析結果報告書

株式会社 三菱化学安全科学研究所
横浜研究所 殿

報告年月日 平成21年2月12日

計量証明事業(濃度)登録茨城県第5号
計量証明事業(音圧)登録茨城県第8号
計量証明事業(振動)登録茨城県第3号
作業環境測定事業登録茨城県第12号株式会社 三菱化学アナリティクス 分析事業所
〒300-0332 茨城県稲敷郡阿見町中央8丁目5番1号
TEL 029 (887) 4511 FAX 029 (887) 5381

御依頼を受けました試料についての分析の結果を次のとおり報告致します。

件 名	水質分析		
試 料 名	B12棟 生物飼育水 (脱塩素水道水)	受付年月日	平成21年2月3日
採取日時	平成21年2月3日 11時40分	試料採取	弊方

注：貴方採取の場合の採取日時はご依頼者の申し出により記入いたしました。

分 析 の 対 象 [単位]	分 析 結 果	基 準 値 等	分 析 の 方 法
Suspended substance (SS) 懸濁物質(SS) [mg/L]	N. D. (< 1.0)	—	JIS.K.0102.14.1 (濾過重量法)
Total Organic Carbon (TOC) 有機体炭素(TOC) [mg/L]	N. D. (< 0.5)	—	JIS.K.0102.22 (燃焼酸化赤外線式分析法)
Biochemical Oxygen Demand (BOD) 有機物(BOD) [mg/L]	0.7	—	JIS.K.0102.21,32.3 (隔膜電極法)
Chemical Oxygen Demand (COD) 有機物(COD) [mg/L]	0.7	—	JIS.K.0102.17 (酸性過マンガン酸-滴定法)
Total Phosphorus 全りん [mg/L]	N. D. (< 0.02)	—	JIS.K.0102.46.3.1 (ペルオキシニ硫酸カリウム分解法)
pH 水素イオン濃度 [—/(℃)]	7.4 (20.0)	—	JIS.K.0102.12.1 (ガラス電極法)
Coliform Group 大腸菌群数 [MPN/100mL]	0	—	S46環告59別表2-1(1) 備考4(最確数法)
Total Mercury 総水銀 [mg/L]	N. D. (< 0.00005)	—	S46環告59号付表1 (還元気化原子吸光法)
Copper 銅 [mg/L]	N. D. (< 0.005)	—	JIS.K.0102.52.5 (ICP質量分析法)
Cadmium カドミウム [mg/L]	N. D. (< 0.001)	—	JIS.K.0102.55.4 (ICP質量分析法)
Zinc 亜鉛 [mg/L]	N. D. (< 0.01)	—	JIS.K.0102.53.4 (ICP質量分析法)
Lead 鉛 [mg/L]	N. D. (< 0.001)	—	JIS.K.0102.54.4 (ICP質量分析法)
Aluminium アルミニウム [mg/L]	0.08	—	JIS.K.0102.58.4 (ICP発光分析法)
Nickel ニッケル [mg/L]	N. D. (< 0.01)	—	JIS.K.0102.59.3 (ICP発光分析法)

分析の対象 [単位]	分析結果	基準値等	分析の方法
Hexavalent Chromium 六価クロム [mg/L]	N. D. (<0.005)	—	JIS.K.0102.65.2.1 (ジフェニルカルバジド吸光法)
Manganese マンガン [mg/L]	N. D. (<0.01)	—	JIS.K.0102.56.5 (ICP質量分析法)
Tin すず [mg/L]	N. D. (<0.03)	—	JIS.K.0102.63.3 (ICP発光分析法)
Silver 銀 [mg/L]	N. D. (<0.01)	—	JIS.K.0102.準拠 (ICP発光分析法)
Cobalt コバルト [mg/L]	N. D. (<0.01)	—	JIS.K.0102.60.3 (ICP発光分析法)
Iron 鉄 [mg/L]	N. D. (<0.04)	—	JIS.K.0102.57.4 (ICP発光分析法)
Total Cyanide 全シアン [mg/L]	N. D. (<0.001)	—	JIS.K.0102.38.1,38.3 (4ピリジンカルボン酸吸光法)
Residual Chlorine 残留塩素 [mg/L]	N. D. (<0.1)	—	JIS.K.0102.33.2 (DPD比色法)
Bromic ion 臭化物イオン [mg/L]	N. D. (<0.5)	—	JIS.K.0102.37.2 (イオンクロマトグラフ法)
Fluorine ふっ素 [mg/L]	N. D. (<0.1)	—	JIS.K.0102.34.1 (ALC-La吸光光度法)
Hydrogen Sulfide 硫化水素 [mg/L]	N. D. (<0.002)	—	S47年環告第9号別表第2 (ガスクロマトグラフ法)
Ammonium nitrogen アンモニア態窒素 [mg/L]	N. D. (<0.2)	—	JIS.K.0102.42.2 (インドフェノール青吸光光度法)
Nitrite nitrogen 亜硝酸態窒素 [mg/L]	N. D. (<0.1)	—	JIS.K.0102.43.1.1 (ナフチルエチレンジアミン法)
Arsenic 砒素 [mg/L]	N. D. (<0.001)	—	JIS.K.0102.61.4 (ICP質量分析法)
Surface-active agents (anionic) 陰イオン界面活性剤 [mg/L]	N. D. (<0.02)	—	JIS.K.0102.30.1.1 (メチレンブルー吸光光度法)
Selenium セレン [mg/L]	N. D. (<0.001)	—	JIS.K.0102.67.4 (ICP質量分析法)
Total Residue 全蒸発残留物 [mg/L]	120	—	JIS.K.0102.14.2 (蒸発乾固-重量法)
Conductivity 電気伝導率 [mS/m]	16	—	JIS.K.0102.13 (電気伝導率計法)
Hardness 全硬度 [mgCaCO3/L]	53	—	JIS.K.0102.50.3,51.3 (ICP発光分析法)
Alkalinity (pH4.8) 酸消費量 (pH4.8) [mgCaCO3/L]	35	—	JIS.K.0102.15.1 (滴定法)
Sodium ナトリウム [mg/L]	8.5	—	JIS.K.0102.48.2 (フレイム原子吸光法)
Potassium カリウム [mg/L]	1.6	—	JIS.K.0102.49.2 (フレイム原子吸光法)
Calcium カルシウム [mg/L]	15	—	JIS.K.0102.50.3 (ICP発光分析法)
Magnesium マグネシウム [mg/L]	4.2	—	JIS.K.0102.51.3 (ICP発光分析法)
Oil (n-Hexane extracts) 油分(ノルマルヘキサン抽出物質)[mg/L]	N. D. (<0.5)	—	JIS.K.0102.24.2 (ヘキサン抽出重量法)

第08E14729号[2] [3/3]

分析の対象 [単位]	分析結果	基準値等	分析の方法
Oil (Oily film/observation) 油分(油膜・外観) [-]	Not recognized	—	JIS.K.0102.8 (外観観察法)
Phenols フェノール類 [mg/L]	N. D. (<0.005)	—	JIS.K.0102.28.1 (吸光光度法)
Polychlorinated Biphenyl (PCB) ポリ塩化ビフェニル(PCB) [mg/L]	N. D. (<0.0005)	—	S46環告59号付表3 (ガスクロマトグラフ法)
Thiram チウラム [mg/L]	N. D. (<0.0006)	—	S46環告59号付表4 (HPLC-UV法)
Simazine シマジン [mg/L]	N. D. (<0.0003)	—	S46環告59号付表5 (GC-NPD法)
Thiobencarb チオベンカルブ [mg/L]	N. D. (<0.002)	—	S46環告59号付表5 (GC-NPD法)
Isoxathion イソキサチオン [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Diazinon ダイアジノン [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Fenitrothion (MEP) フェントロチオン(MEP) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Isoprothiolane イソプロチオラン [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Oxine-copper オキシ銅 [mg/L]	N. D. (<0.001)	—	H5環水規121号付表2 (HPLC法)
Chlorothalonil (TPN) クロタロニル (TPN) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Propyzamide プロピザミド [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
EPN EPN [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Dichlorovos (DDVP) ジクロロボス (DDVP) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Fenobucarb (BPMC) フェノブカルブ (BPMC) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Iprobenfos (IBP) イプロベンホス (IBP) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
Chloronitofen (CNP) クロロニトロフェン (CNP) [mg/L]	N. D. (<0.001)	—	H5環水規121号付表1 (GC-MS法)
—以下余白—			
[備考] 水産用水基準 (2005)			

Appendix 3

Acute toxicity test

(3 pages)

Acute Toxicity Test

In order to confirm that the concentration levels of bioconcentration test were less than a hundredth and a thousandth of the 96-hour LC_{50} to Medaka, the acute toxicity test was performed.

1 Test Methods

The test was conducted according to the OECD Guidelines for Testing of Chemicals No.203 "Fish, acute Toxicity Test" (1992). The concentration of the test substance was not measured, and LC_{50} was evaluated based on the nominal concentration.

1.1. Test Conditions

Exposure period:	96 hours
Concentration level:	0 (control), 100 mg/L (Limit test)
Number of fish:	10 / group
Volume of test water:	5.0 L
Water temperature:	24 ± 1 °C
Test mode:	Semi-static
	Renewal of test water: Every 24 hours
	Cover each vessel with a plastic plate
	Light (Approximately 16 hr/day)
Aeration:	Continuous
Dissolved oxygen:	$\geq 60\%$ of the saturation (≥ 5 mg/L at 24 °C)
Feeding:	None

1.2. Preparation of the test water

1) For exposure group

Zero point five grams of the test substance was dissolved in 5 L with dilution water.

2) For control

Only dilution water was used.

4.4. Results

The 96-hour LC₅₀ of the test substance was >100 mg/L.

Concentration of test substance (mg/L)	Cumulative-mortality (%)			
	24 hr	48 hr	72 hr	96 hr
0	0	0	0	0
100	0	0	0	0

The nominal concentration of bioconcentration test was confirmed to be less than a hundredth and a thousandth of the 96-hour LC₅₀.

Appendix 4

Calibration curve and chromatograms (Standard solution, blank test and recovery test)

(20pages)

Figure 1 Calibration curve of the test substance

Input Data

No.	Concentration (mg/L)	Peak Area (count)
1	0	0
2	0.01	796.6
3	0.02	1437.2
4	0.04	2899.1

$$Y = 72,700X$$

$$r = 0.9998$$

detection limit = 0.001mg/L

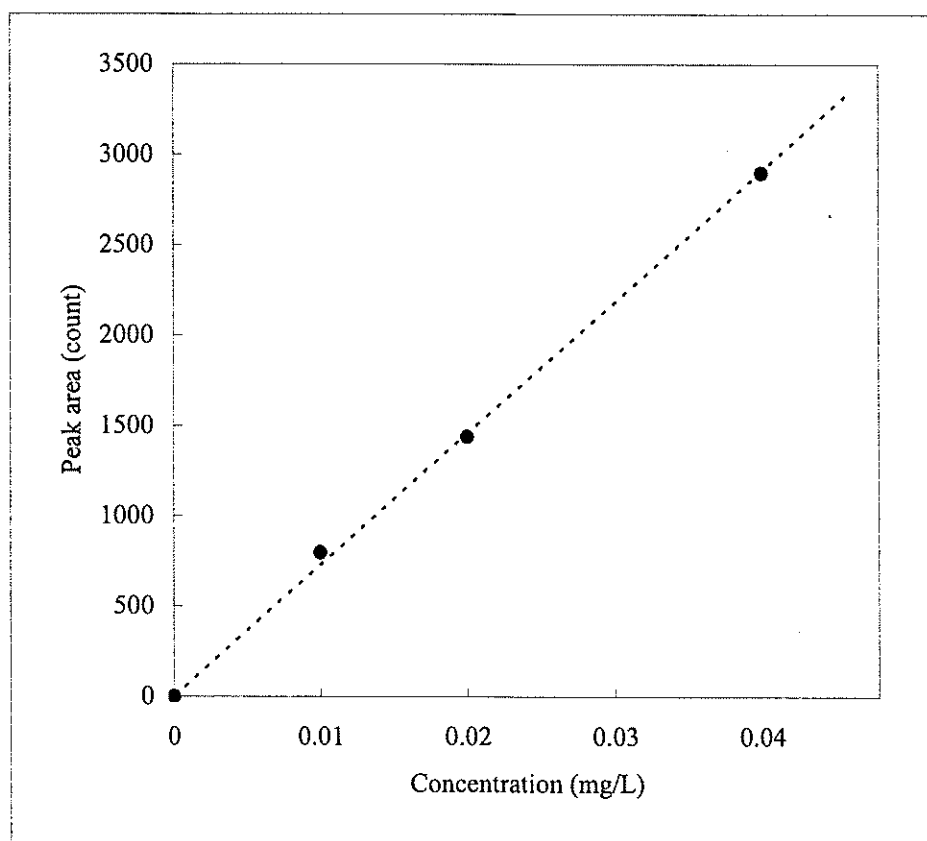


Figure 2 Chromatograms of the test substance – standard solutions

Std. 0 mg/L

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 08 Apr 2009 18:15:22

Date: 08-Apr-2009

Time: 18:05:04

Description: std 0mg/L

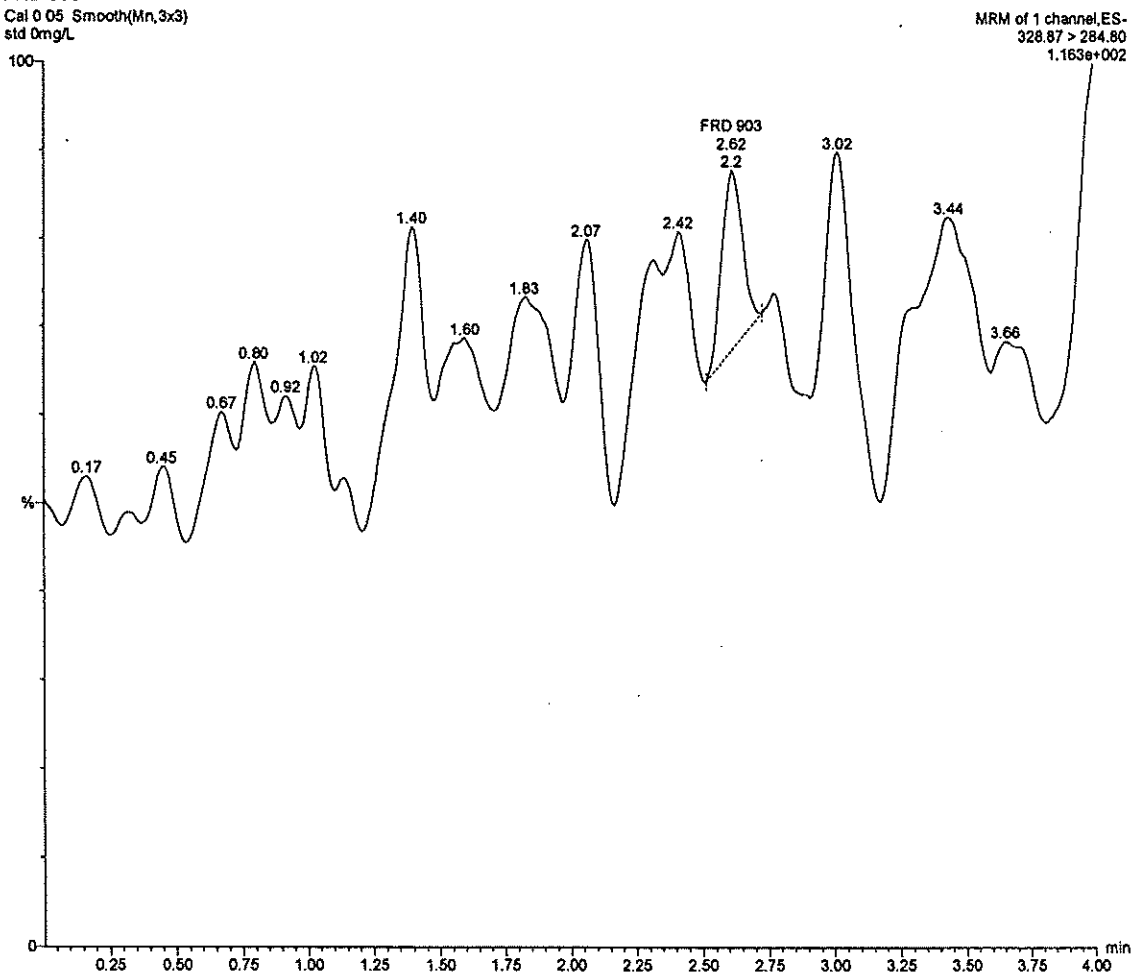
Name: Cal 0 05

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\BIA080560_02

Vial: 1:B,1

User: 志

FRD 903

Cal 0 05 Smooth(Mn,3x3)
std 0mg/L

#	Name	RT	Area	Height
1	FRD 903			

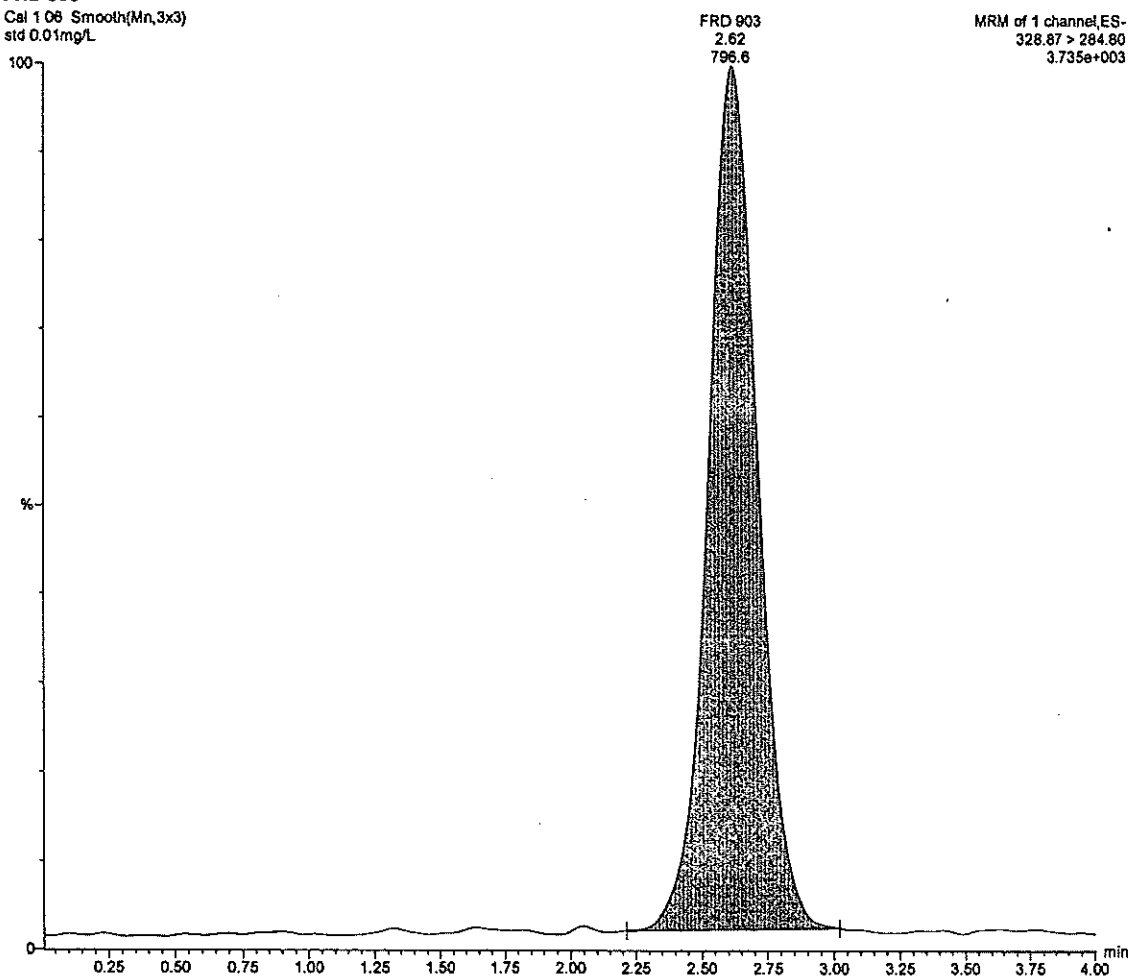
Figure 2 Chromatograms of the test substance – standard solutions (continued)

Std. 0.01 mg/L

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Date: 08-Apr-2009
Time: 15:39:08
Description: std 0.01mg/L
Name: Cal 1 06
Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,7
User: 志

FRD 903
Cal 1 06 Smooth(Mn,3x3)
std 0.01mg/L



#	Name	RT	Area	Height
1	FRD 903	2.618	796.6	3647

Figure 2 Chromatograms of the test substance – standard solutions (continued)

Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 08-Apr-2009

Time: 15:44:43

Description: std 0.02mg/L

Name: Cal 2 06

Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,4

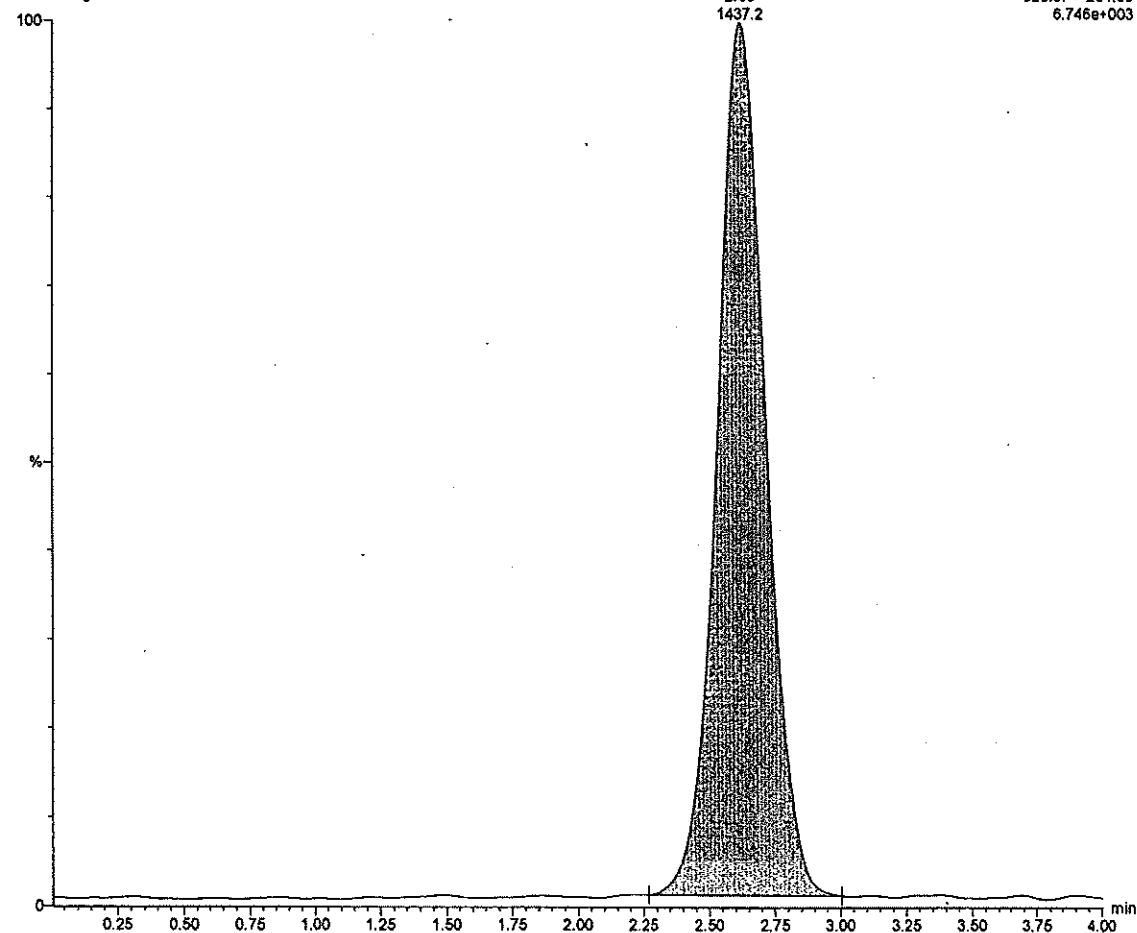
User: 志

FRD 903Cal 2 06 Smooth(Mn,3x3)
std 0.02mg/L

FRD 903

2.63

1437.2

MRM of 1 channel, ES-
328.87 > 284.80
6.746e+003

#	Name	RT	Area	Height
1	FRD 903	2.627	1437.2	6659

Figure 2 Chromatograms of the test substance – standard solutions (continued)

Std. 0.04 mg/L

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

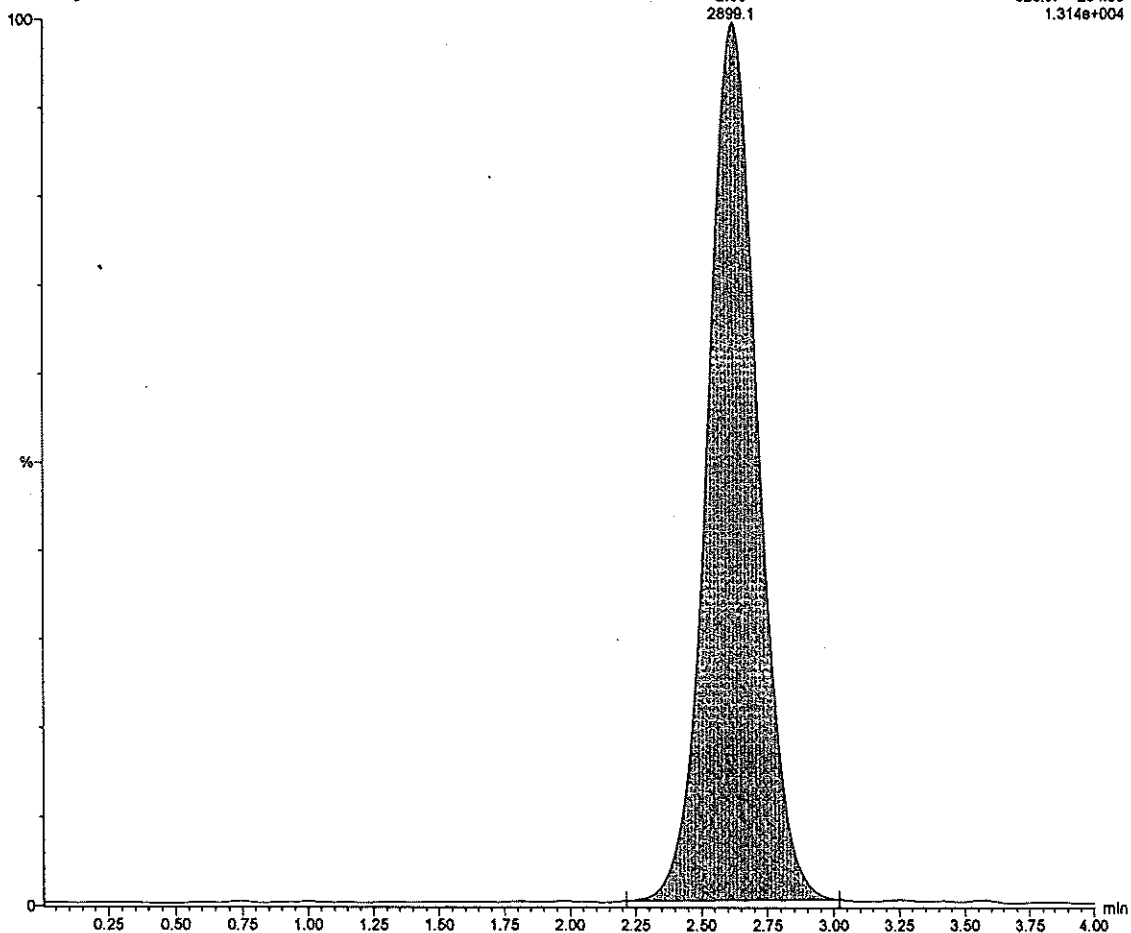
Date: 08-Apr-2009
Time: 15:50:14
Description: std 0.04mg/L
Name: Cal 3 06
Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,5
User: KS

FRD 903

Cal 3 06 Smooth(Mn,3x3)
std 0.04mg/L

FRD 903
2.63
2899.1

MRM of 1 channel, ES-
328.87 > 284.80
1.314e+004



Peak	Name	RT	Area	Height
1	FRD 903	2.627	2899.1	13035

Figure 3 Chromatograms of blank test of the test substance in water
Std. 0.02mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 08 Apr 2009 17:56:15

Date: 08-Apr-2009

Time: 17:26:54

Description: std 0.02mg/L

Name: 09040822

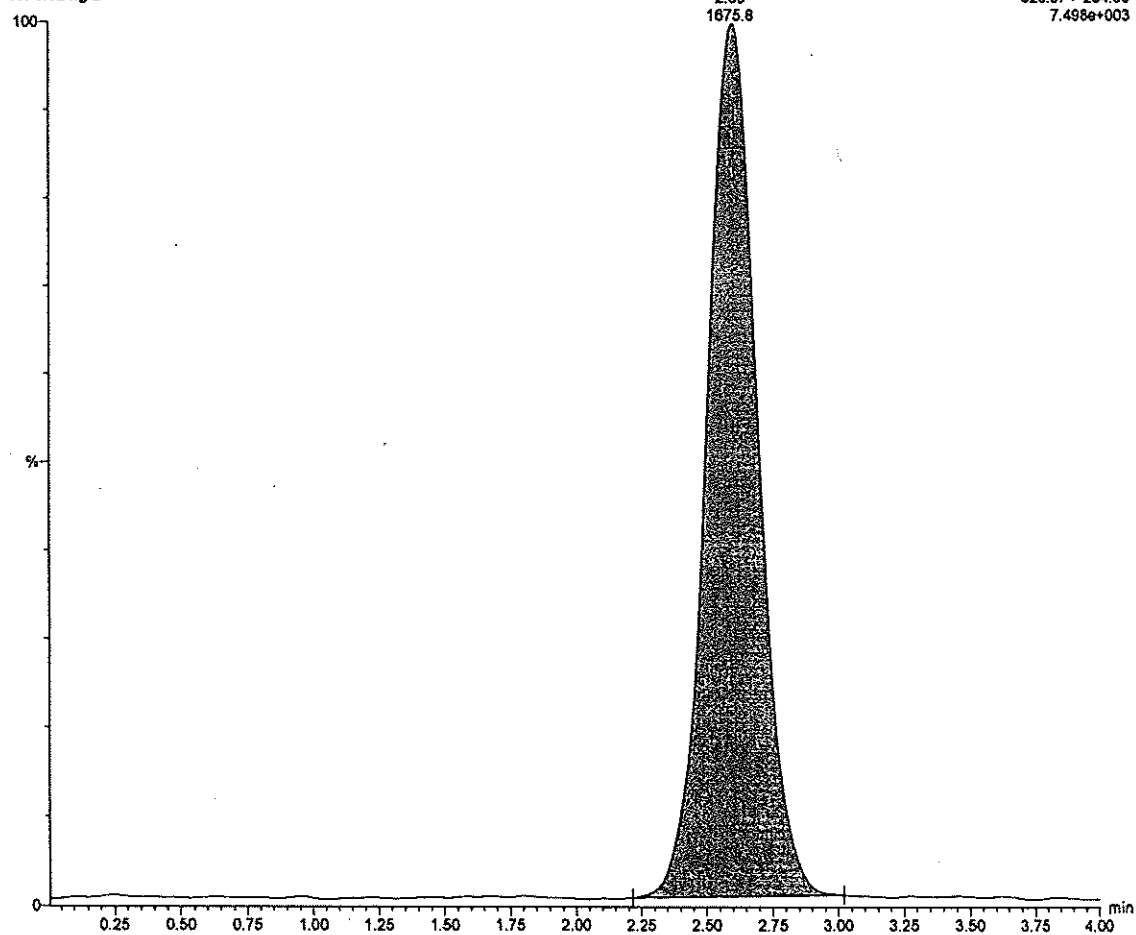
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A.4

User: 志

FRD 903

09040822 Smooth(Mn,3x3)
std 0.02mg/L



MRM of 1 channel, ES-
328.87 > 284.80
7.498e+003

Peak	Name	RT	Area	Height
1	FRD 903	2.601	1675.8	7414

Figure 3 Chromatograms of blank test of the test substance in water (continued)

Sample (No.1)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 08-Apr-2009

Time: 17:48:28

Description: WB1

Name: WB 1 02

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,2

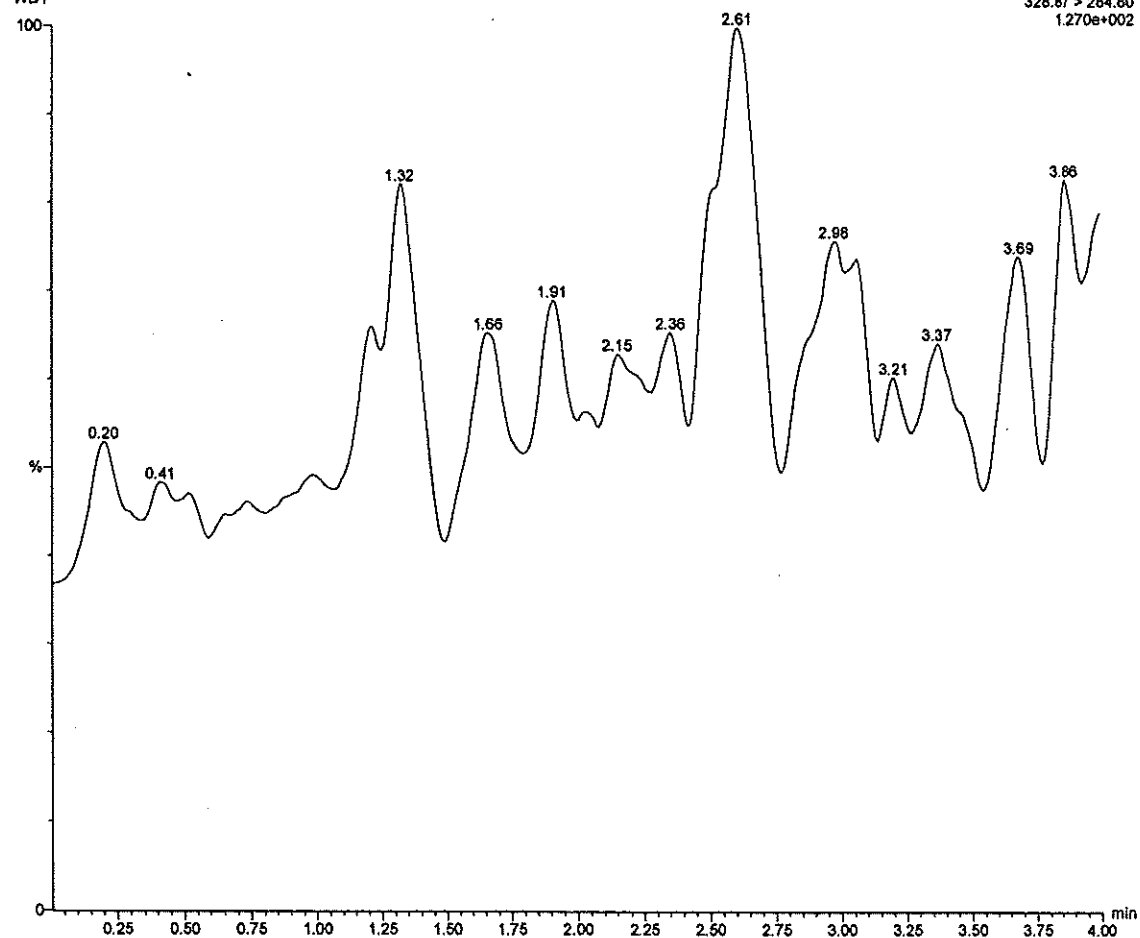
User: 志

FRD 903

WB 1 02 Smooth(Mn,3x3)

WB1

MRM of 1 channel, ES-
328.87 > 284.80
1.270e+002



Peak	Name	Area	Height
1	FRD 903		

Figure 3 Chromatograms of blank test of the test substance in water (continued)

Sample (No.2)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 08-Apr-2009

Time: 17:37:21

Description: WB2

Name: WB 2 01

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PROVACQUDB\A080560_02

Vial: 1:B,3

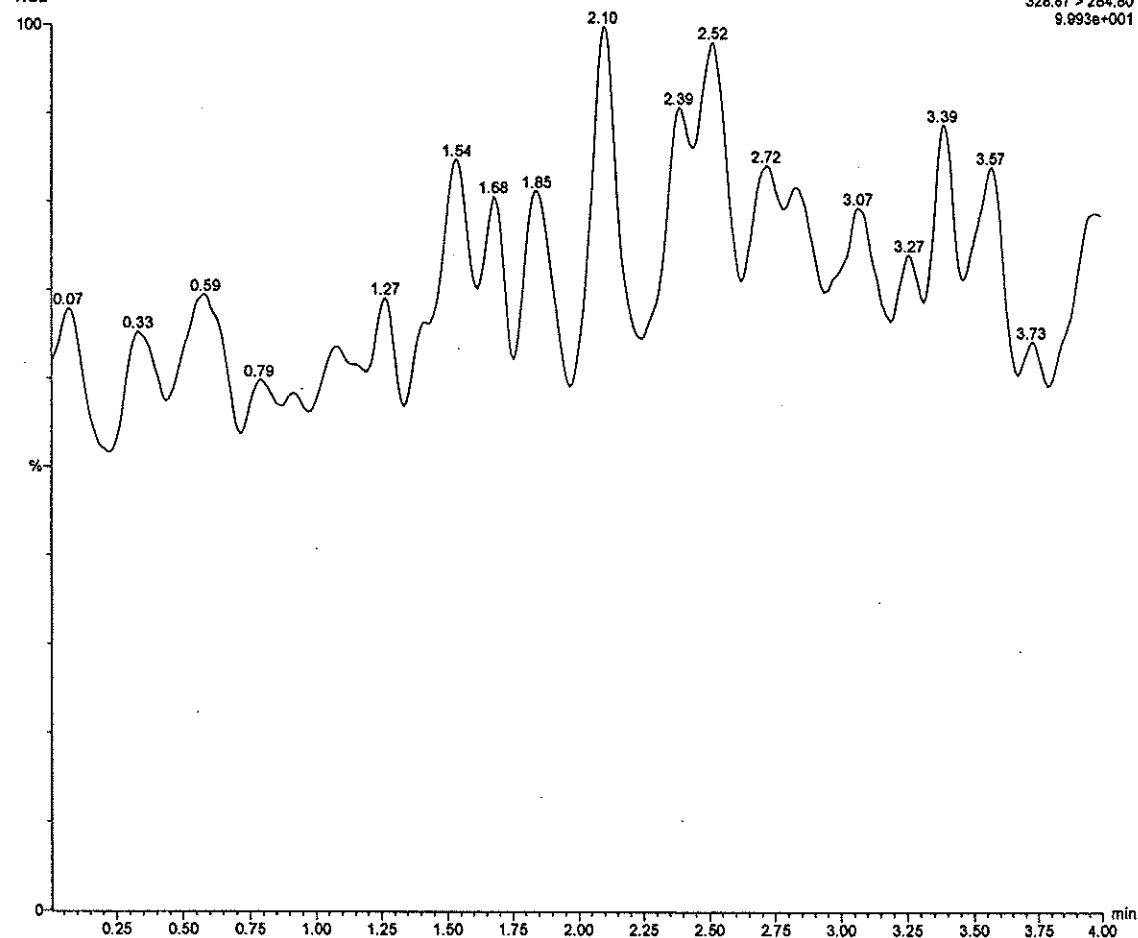
User: 志

FRD 903

WB 2 01 Smooth(Mn,3x3)

WB2

MRM of 1 channel, ES-
328.87 > 284.80
9.993e+001



1	Name	RT	Area	Height
1	FRD 903			

Figure 4 Chromatograms of blank test of the test substance in fish
Std. 0.02 mg/L, before the start of the exposure

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PROMethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 13 Apr 2009 19:27:31

Date: 13-Apr-2009

Time: 18:29:05

Description: std 0.02mg/L

Name: S041307

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PROVACQUDB\A080560_02

Vial: 1:F,3

User: *KG*

FRD 903

S041307 Smooth(Mn,3x3)
std 0.02mg/L

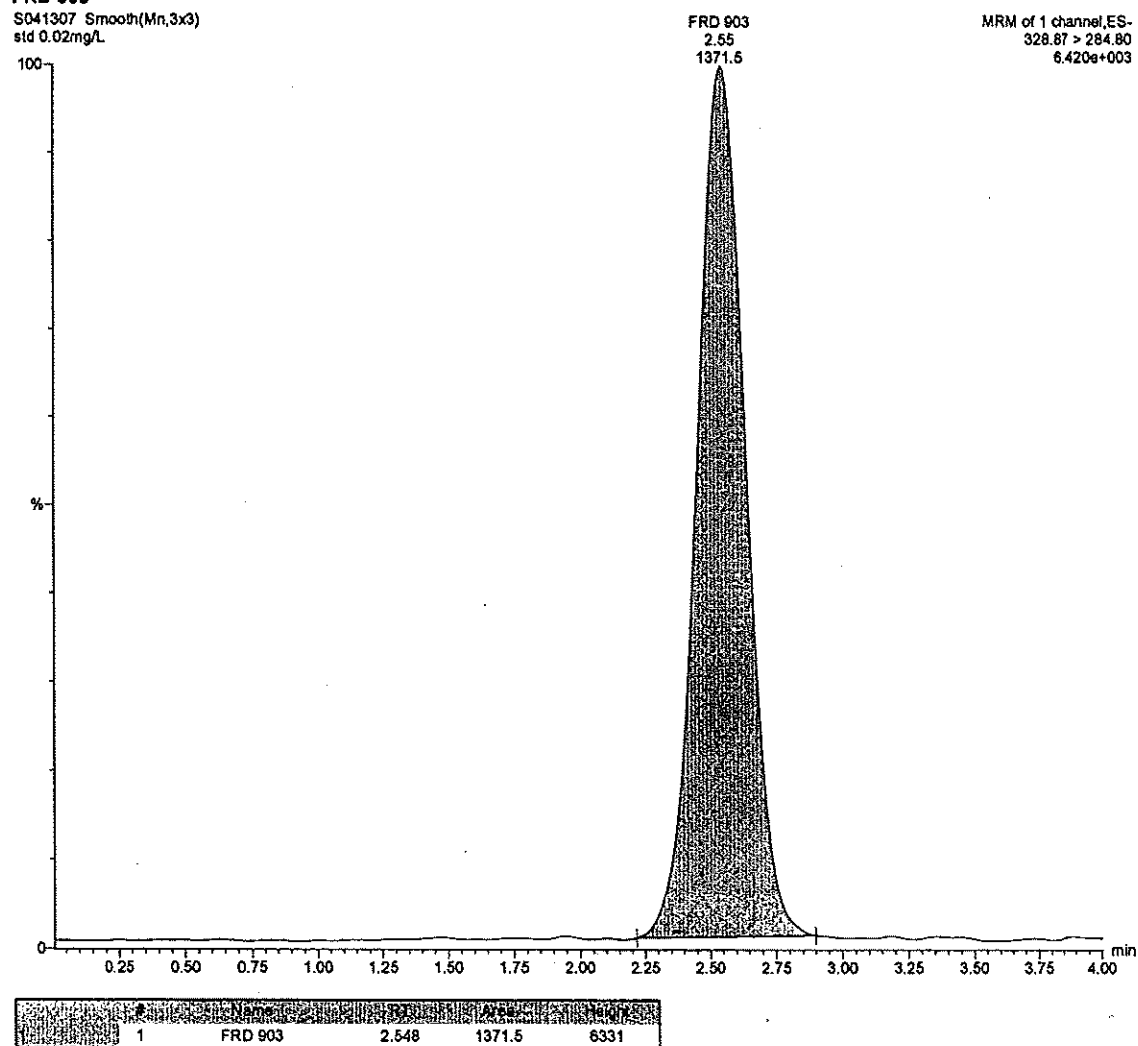


Figure 5 Chromatograms of blank test of the test substance in fish (continued)

Sample (No.1) , before the start of the exposure

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Date: 13-Apr-2009


Time: 18:34:37

Description: FB1

Name: FB1 01

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

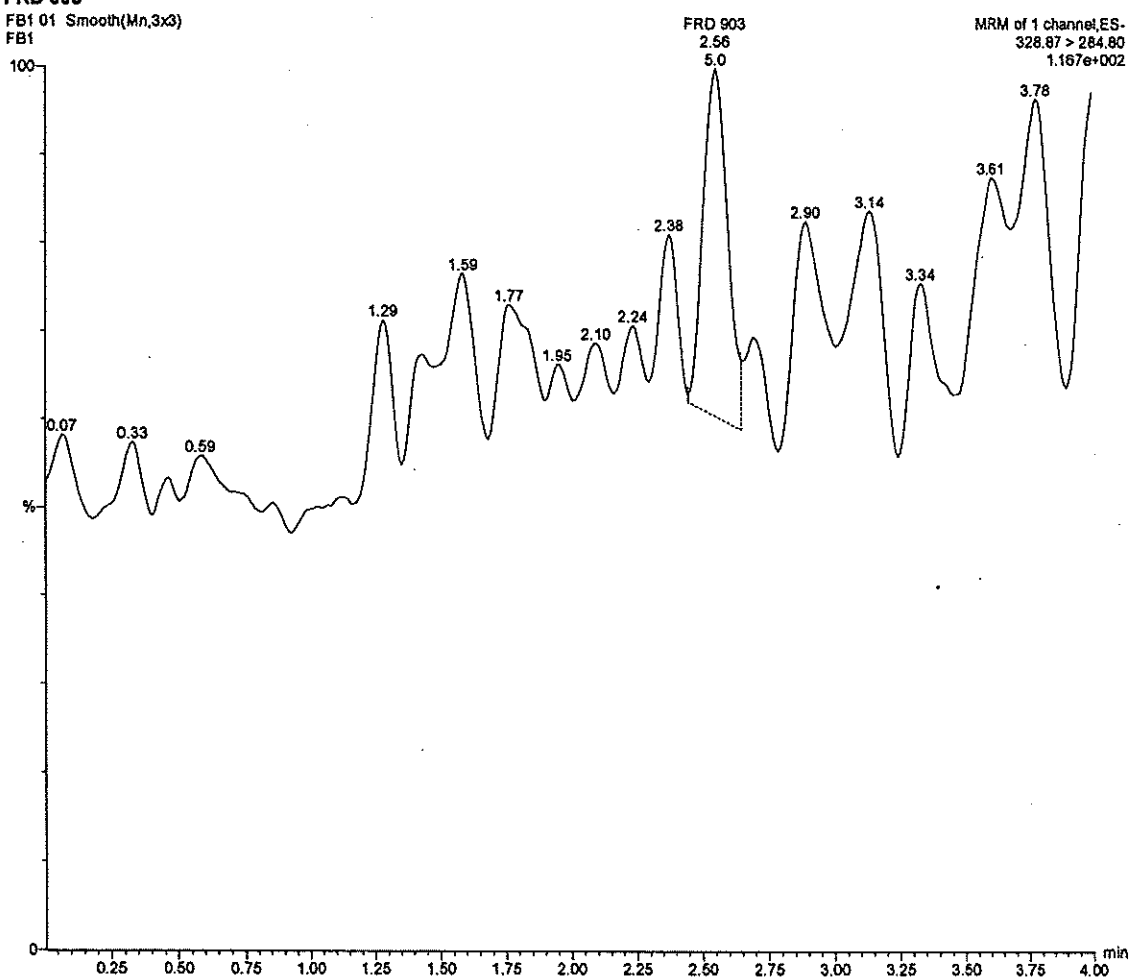
Vial: 1:F,5

User: 

FRD 903

FB1 01 Smooth(Mn,3x3)

FB1



#	Name	RT	Area	Height
1	FRD 903			

Figure 6 Chromatograms of blank test of the test substance in fish (continued)

Sample (No.2) , before the start of the exposure

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 13-Apr-2009

Time: 18:40:08

Description: FB2

Name: FB2 01

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

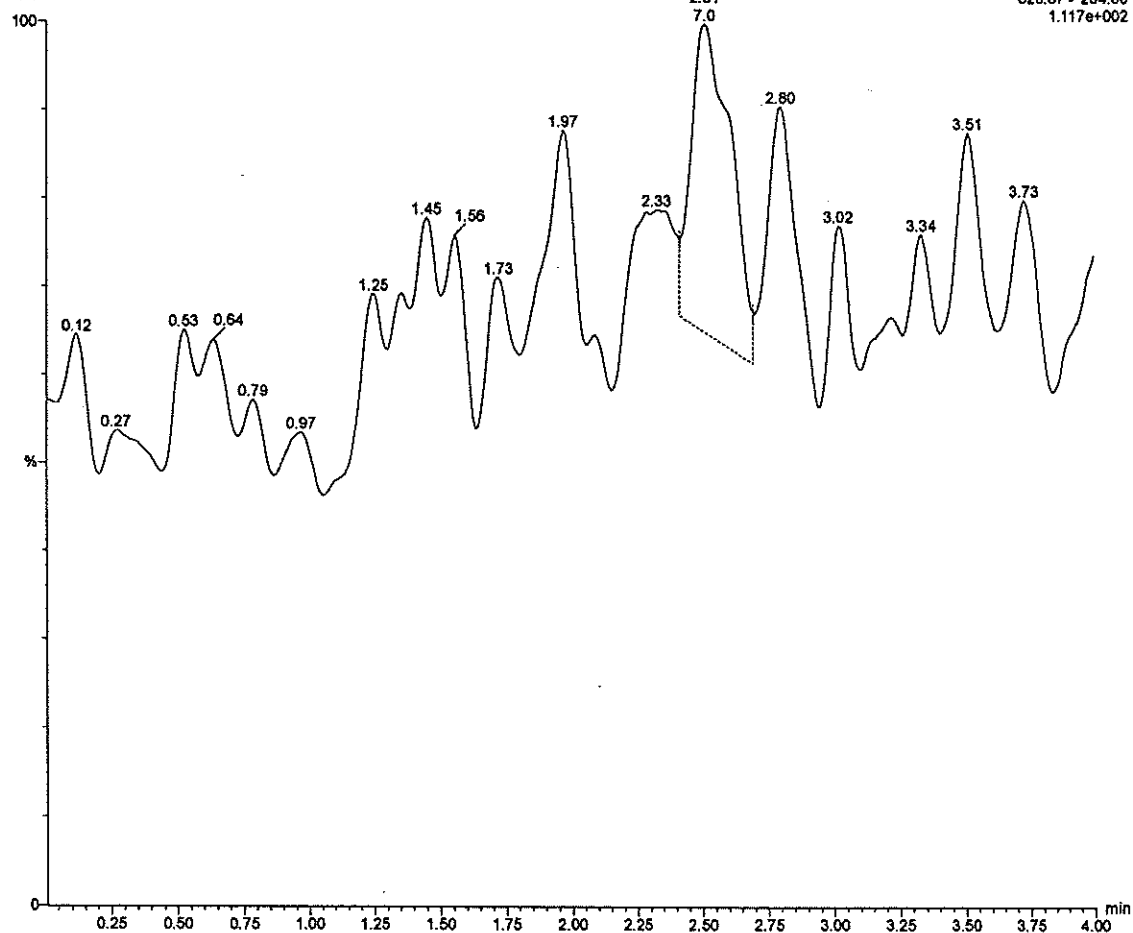
Vial: 1:F,6

User: 志

FRD 903

FB2 01 Smooth(Mn,3x3)

FB2



#	Name	RT	Area	Height
1	FRD 903			

Figure 7 Chromatograms of blank test of the test substance in fish (continued)

Std. 0.02 mg/L, at the end of the exposure

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 18 May 2009 18:22:03

Date: 18-May-2009

Time: 15:19:19

Description: std 0.02mg/L

Name: S051804

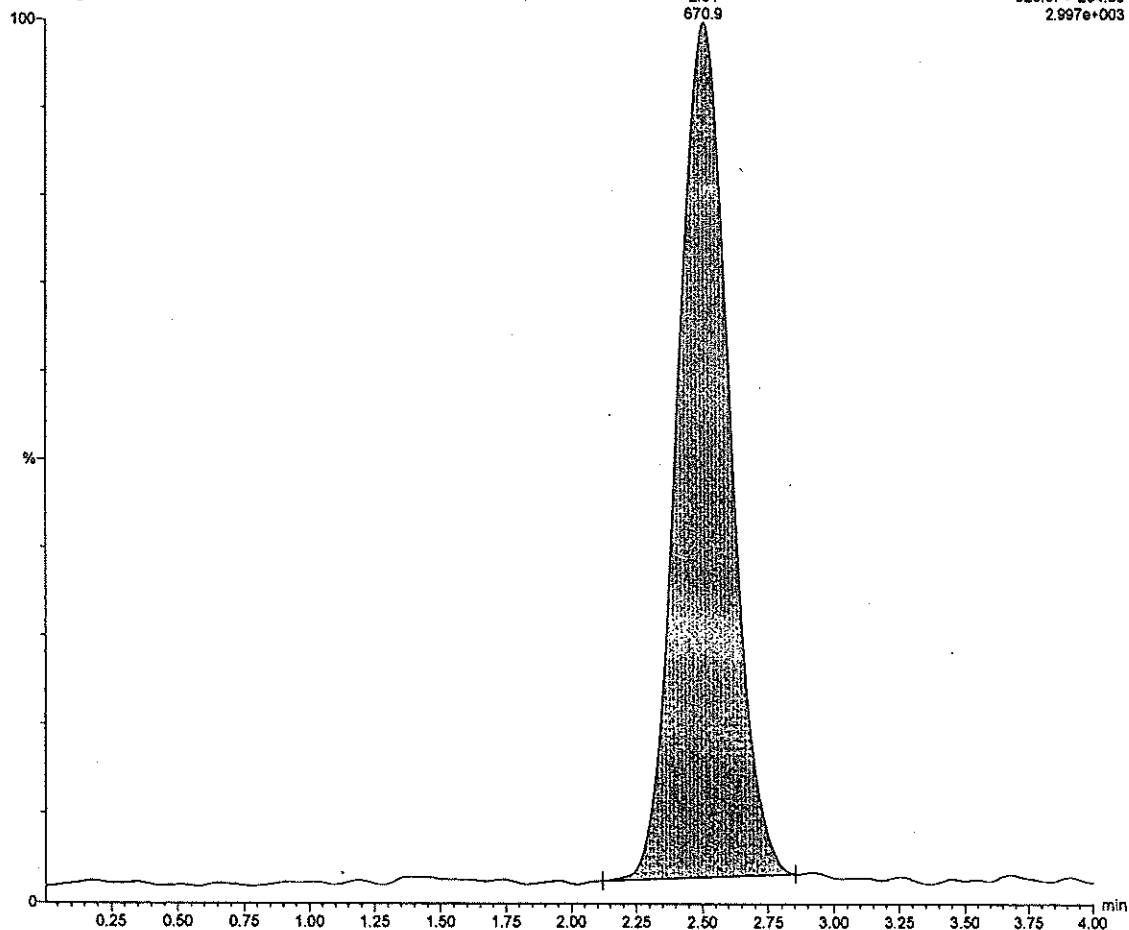
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,4

User: 志

FRD 903

S051804 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.513	670.9	2910

Figure 8 Chromatograms of blank test of the test substance in fish (continued)

Sample (No.3) , at the end of the exposure

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 18-May-2009

Time: 15:24:53

Description: FB3

Name: FB302

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

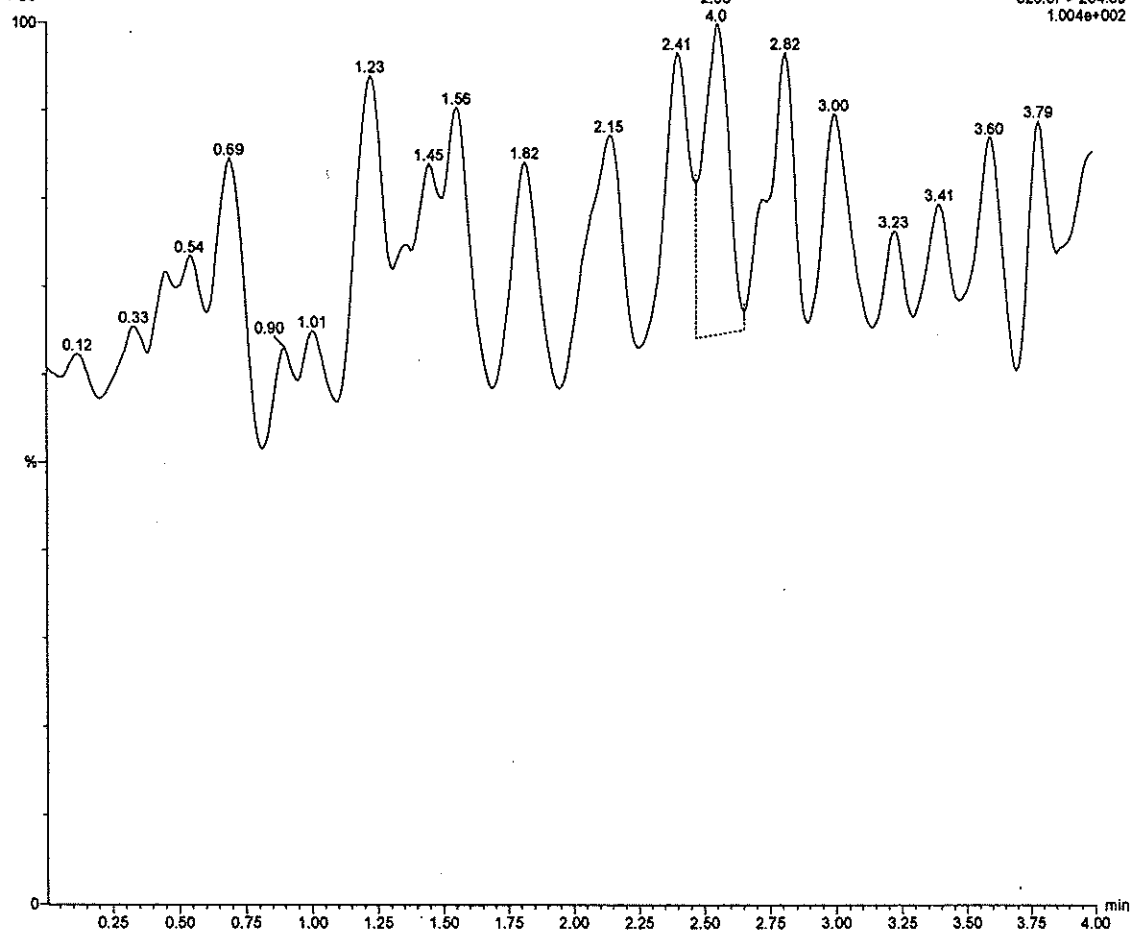
Vial: 1:A,5

User: *is*

FRD 903

FB302 Smooth(Mn,3x3)

FB3



#	Name	RT	Area	Height
1	FRD 903			

Figure 9 Chromatograms of blank test of the test substance in fish (continued)

Sample (No.4) , at the end of the exposure

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 18-May-2009

Time: 15:30:23

Description: FB4

Name: FB402

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

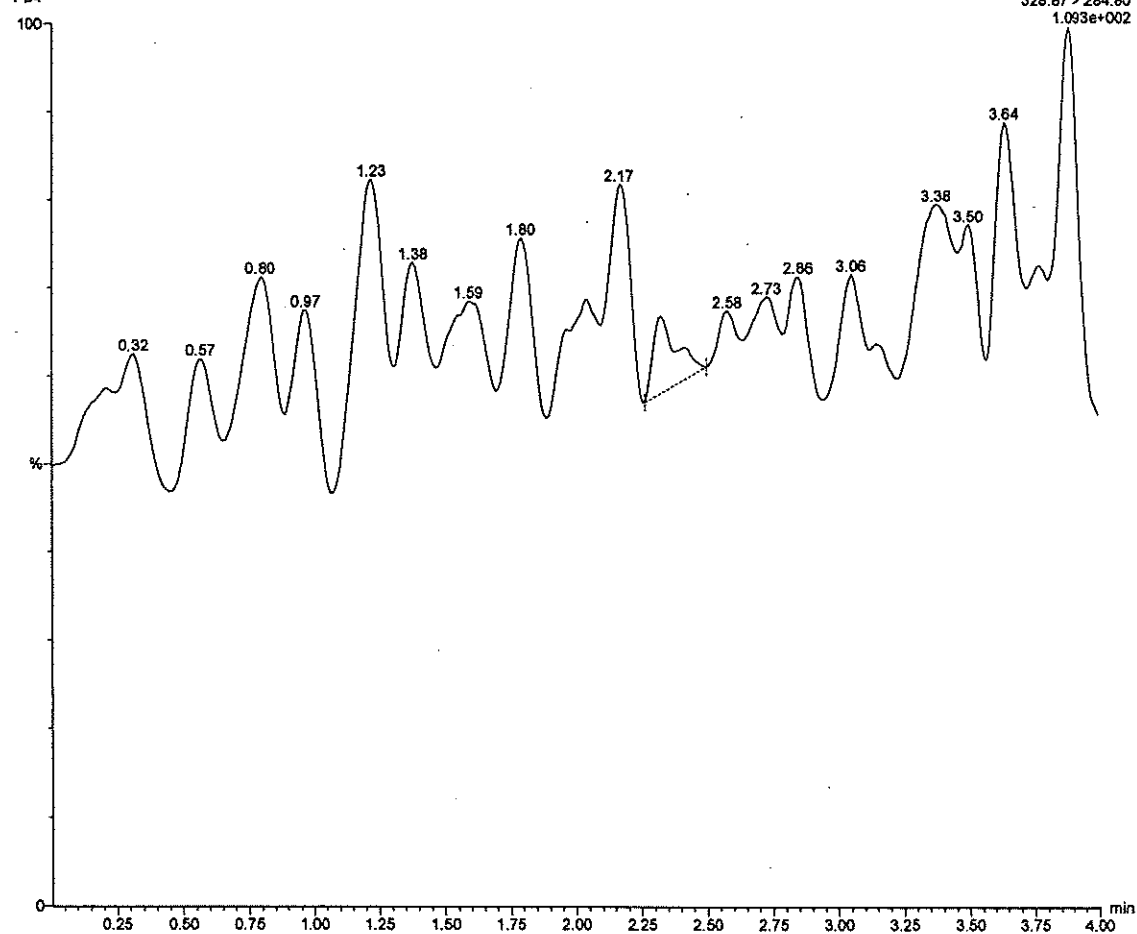
Vial: 1:A,6

User: 志

FRD 903

FB402 Smooth(Mn,3x3)

FB4



#	Name	RT	Area	Height
1	FRD 903			

Figure 10 Chromatograms of recovery test of the test substance in fish
Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 10-Apr-2009

Time: 20:33:31

Description: std 0.02mg/L

Name: S041007

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

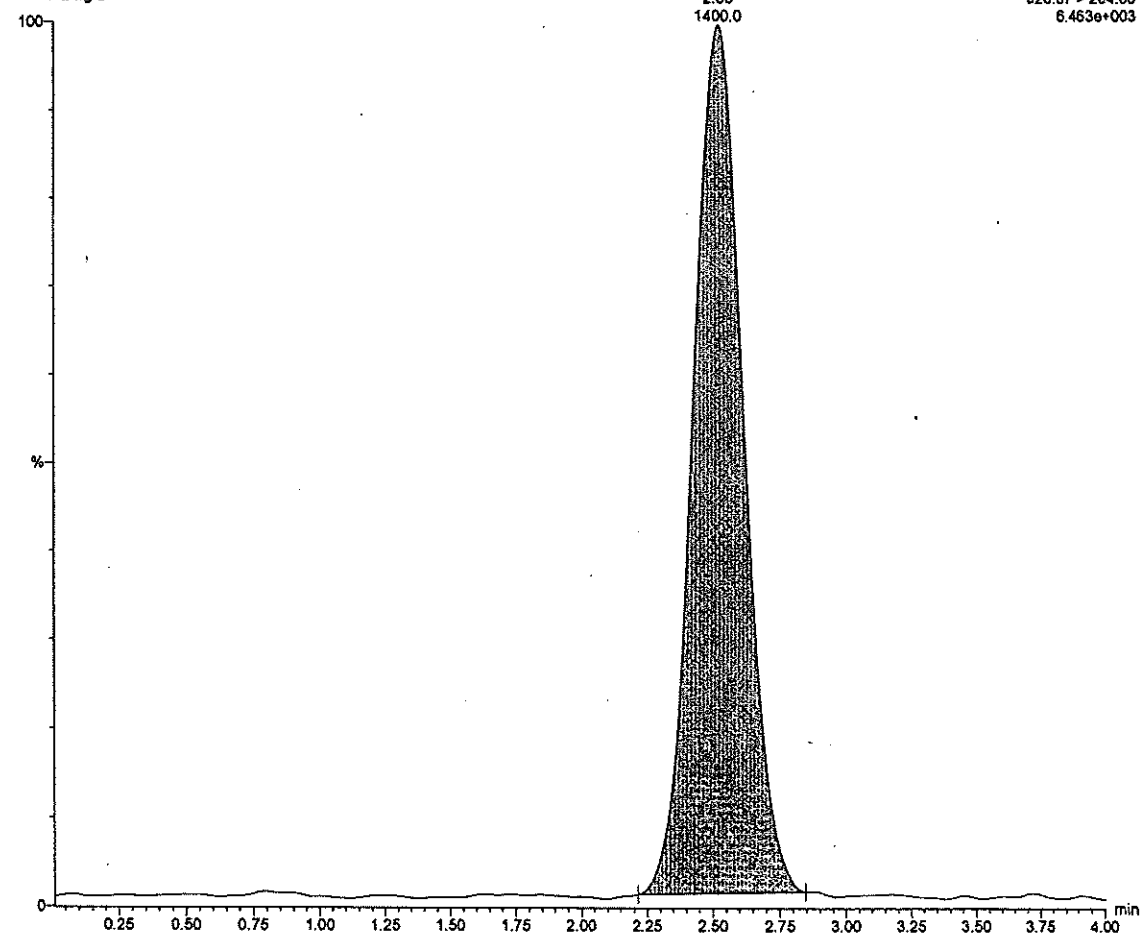
User: *ky*

FRD 903

S041007 Smooth(Mn,3x3)
std 0.02mg/L

FRD 903
2.53
1400.0

MRM of 1 channel, ES-
328.87 > 284.80
6.463e+003



#	Name	RT	Area	Height
1	FRD 903	2.531	1400.0	6358

Figure 6 Chromatograms of recovery test of the test substance in fish (continued)

Sample (No.1)

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PROMethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 10 Apr 2009 21:31:31

Date: 10-Apr-2009

Time: 20:38:32

Description: FR1

Name: FR1 05

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

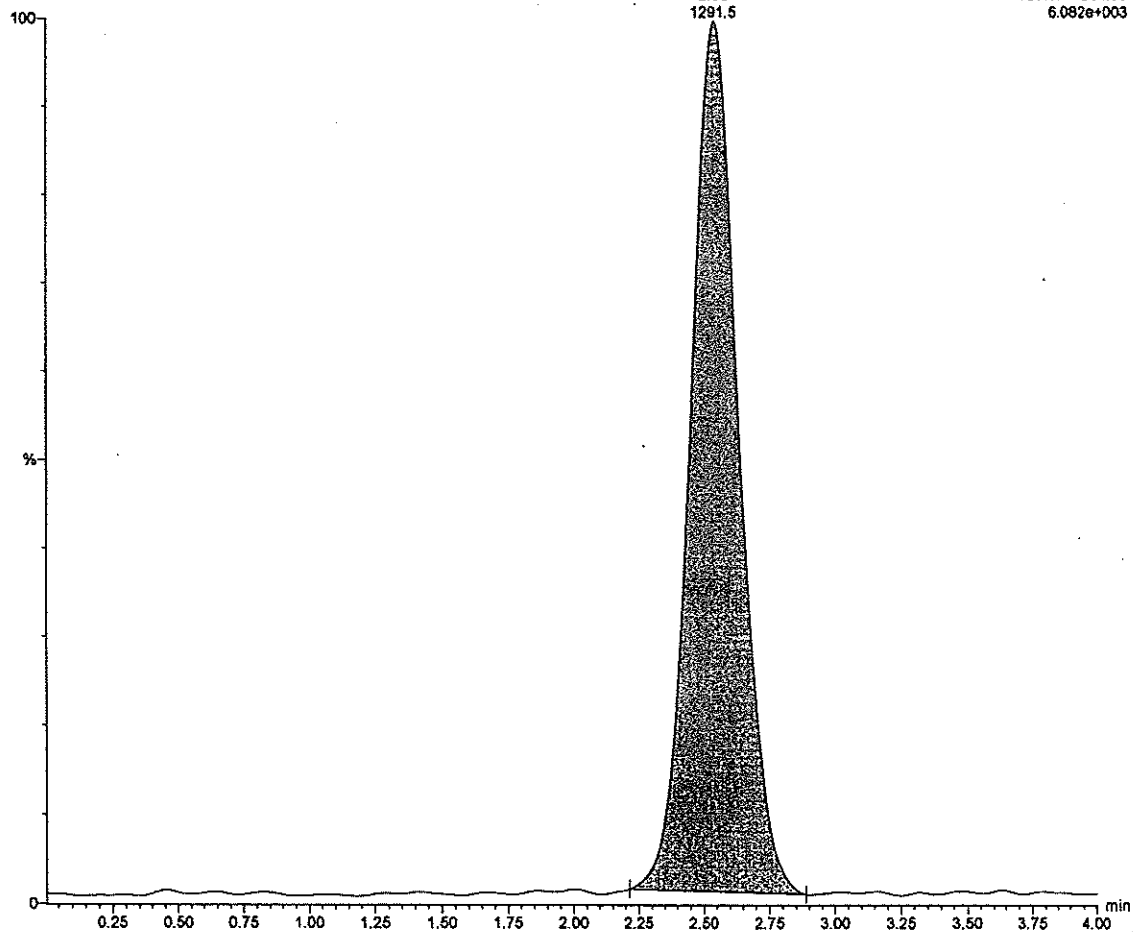
Vial: 1:A,4

User: 天

FRD 903

FR1 05 Smooth(Mn,3x3)

FR1



#	Name	RT	Area	Height
1	FRD 903	2.548	1291.5	5993

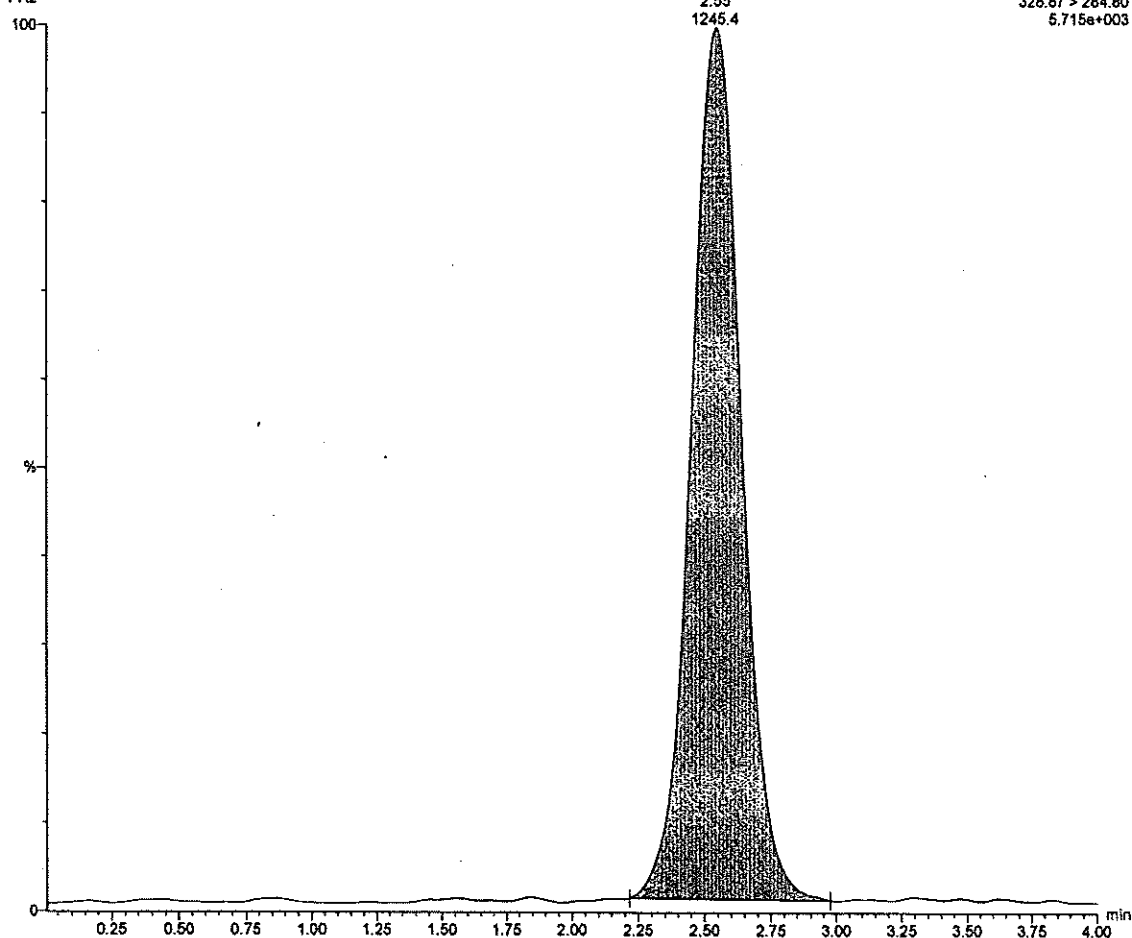
Figure 6 Chromatograms of recovery test of the test substance in fish (continued)
Sample (No.2)

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Date: 10-Apr-2009
Time: 20:43:59
Description: FR2
Name: FR2 05
Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,5
User: 

FRD 903

FR2 05 Smooth(Mn,3x3)
FR2



#	Name	RT	Area	Height
1	FRD 903	2.548	1245.4	5630

Figure 6 Chromatograms of recovery test of the test substance in fish (continued)
Sample (No.3)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 10-Apr-2009

Time: 20:49:31

Description: FR3

Name: FR3 05

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDE\A080560_02

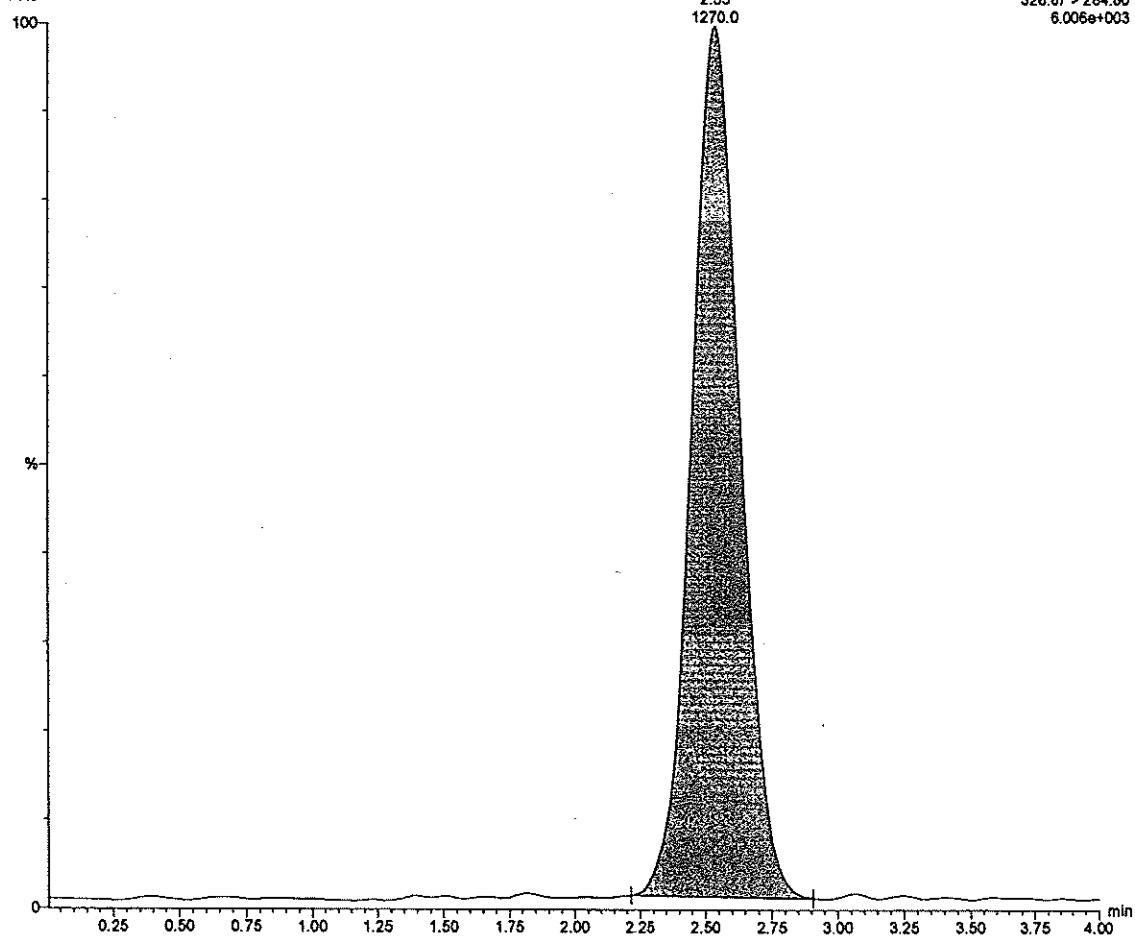
Vial: 1:A,6

User: 志

FRD 903

FR3 05 Smooth(Mn,3x3)

FR3



#	Name	RT	Area	Height
1	FRD 903	2.548	1270.0	5922

Figure 6 Chromatograms of recovery test of the test substance in fish (continued)
Sample (No.4)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 10-Apr-2009

Time: 20:55:05

Description: FR4

Name: FR4 05

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PROVACQUDB\A080560_02

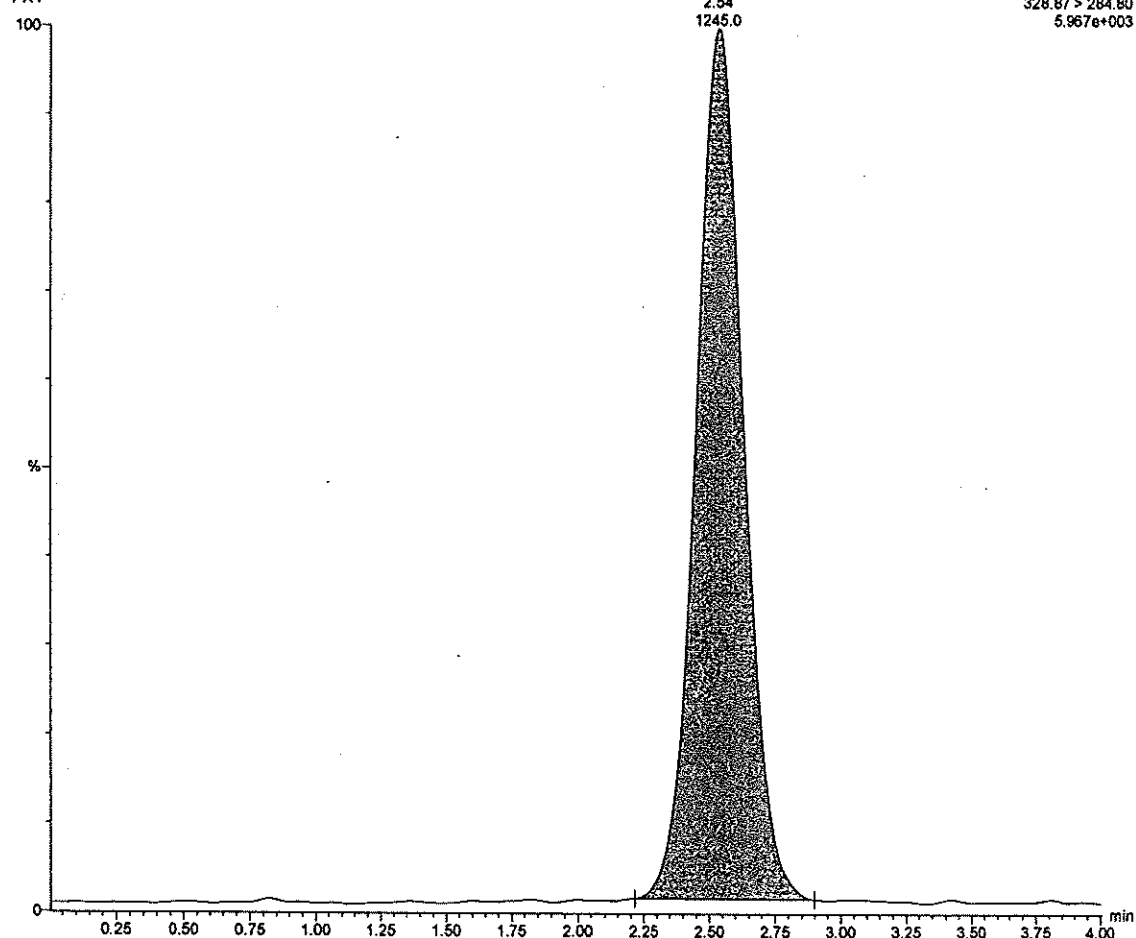
Vial: 1:A,7

User: 天

FRD 903

FR4 05 Smooth(Mn,3x3)

FR4



#	Name	RT	Area	Height
1	FRD 903	2.540	1245.0	5869

Appendix 5

Chromatograms (Analysis of the test substance in the test water)

(19 pages)

Figure 1 Chromatograms of test water analysis
day 0, Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 16 Apr 2009 13:14:46

Date: 16-Apr-2009


Time: 12:50:14

Description: std 0.02mg/L

Name: S041610

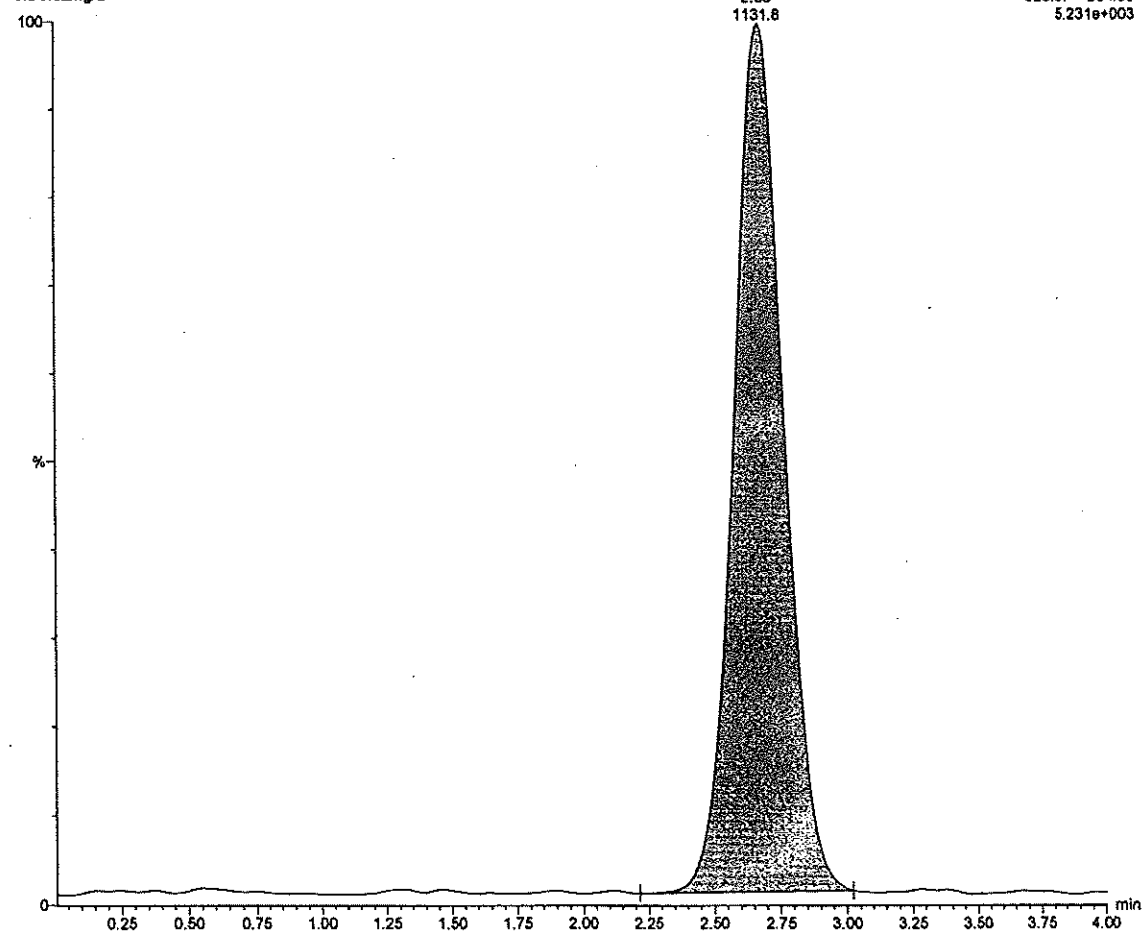
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: 

FRD 903

S041610 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.680	1131.8	5155

Figure 2 Chromatograms of test water analysis (continued)
day 0, High concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 16-Apr-2009

Time: 13:01:18

Description: WH0D

Name: WH0D04

Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02

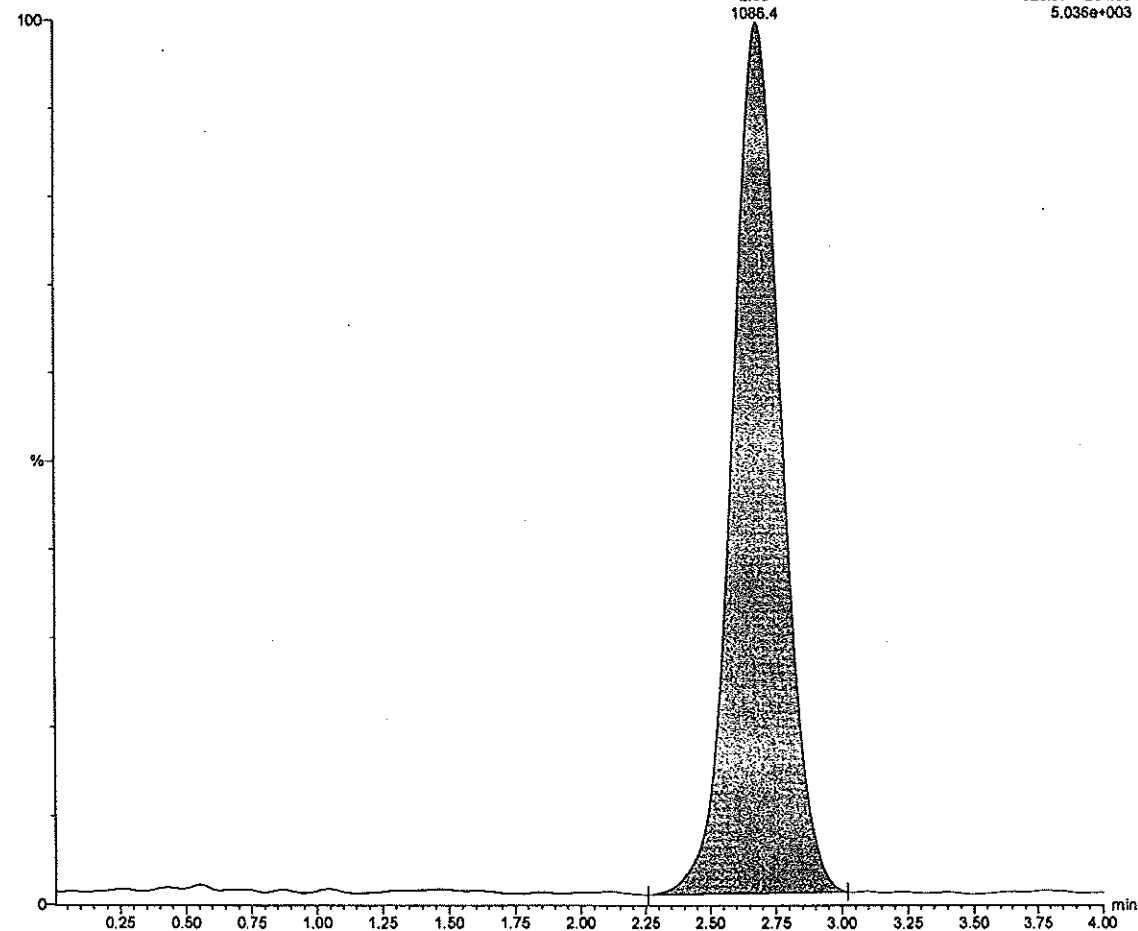
Vial: 1:A,5

User: *ts*

FRD 903

WH0D04 Smooth(Mn,3x3)

WH0D



#	Name	RT	Area	Height
1	FRD 903	2.689	1086.4	4971

Figure 3 Chromatograms of test water analysis (continued)
day 0, Low concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 16-Apr-2009

Time: 12:55:50

Description: WL0D

Name: WL0D04

Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A.4

User: *LS*

FRD 903

WL0D04 Smooth(Mn,3x3)

WL0D

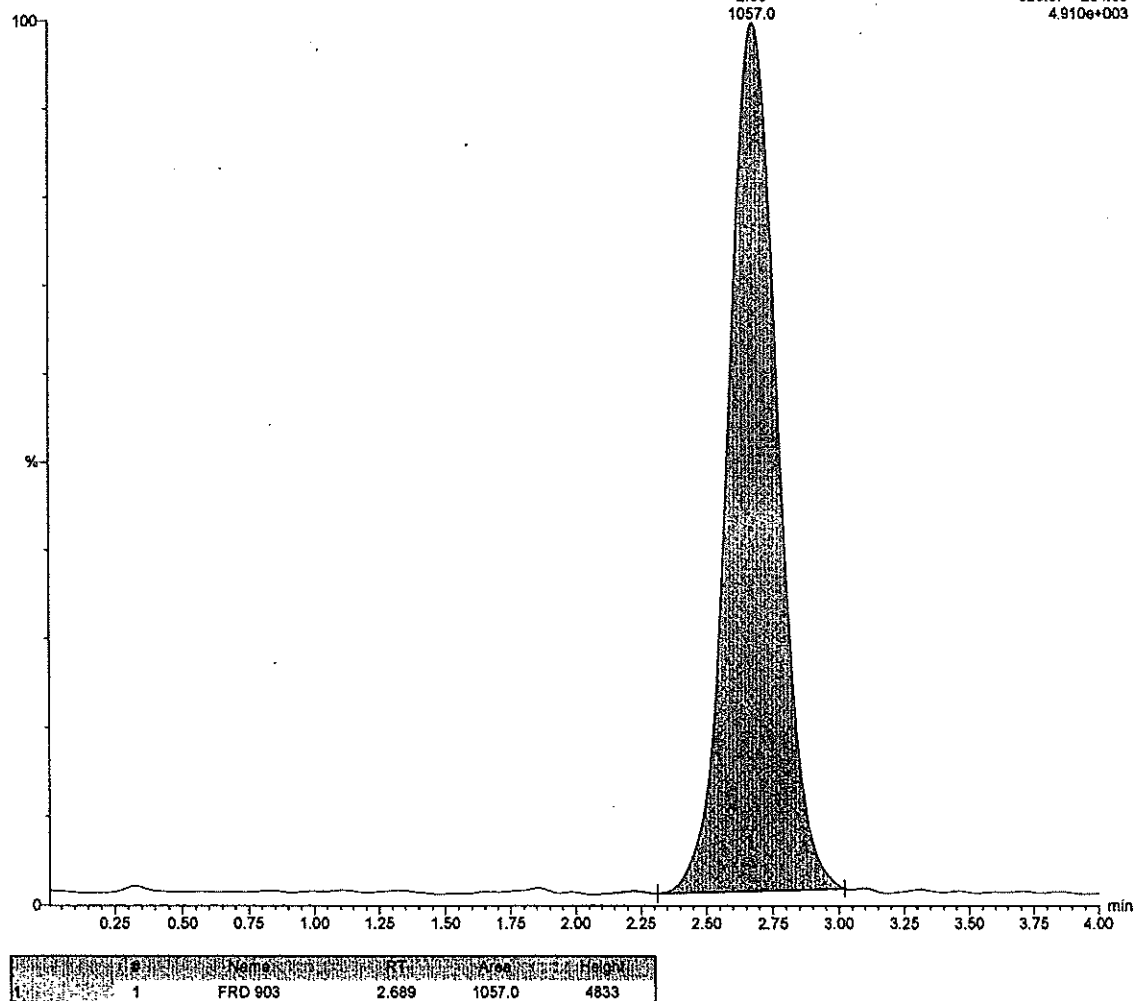


Figure 4 Chromatograms of test water analysis (continued)

day 4, Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903**MassLynx 4.1**

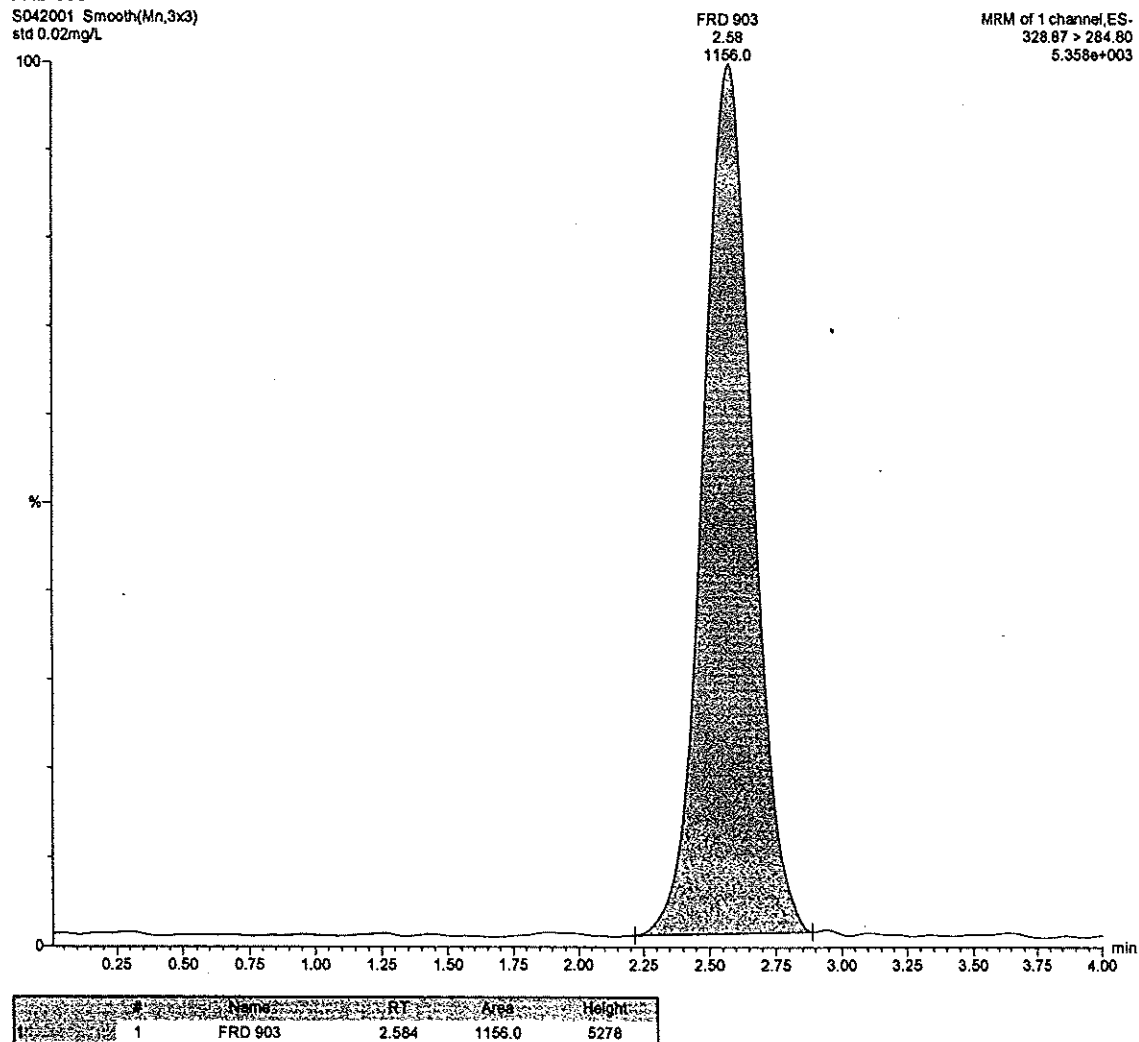
Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 20 Apr 2009 11:28:09**Date:** 20-Apr-2009**Time:** 10:49:48**Description:** std 0.02mg/L**Name:** S042001**Inlet Method Name:** C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02**Vial:** 1:A,3**User:** 志**FRD 903**S042001 Smooth(Mn,3x3)
std 0.02mg/L

Figure 5 Chromatograms of test water analysis (continued)
day 4, High concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009

Time: 11:00:50

Description: WH4D

Name: WH4D01

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

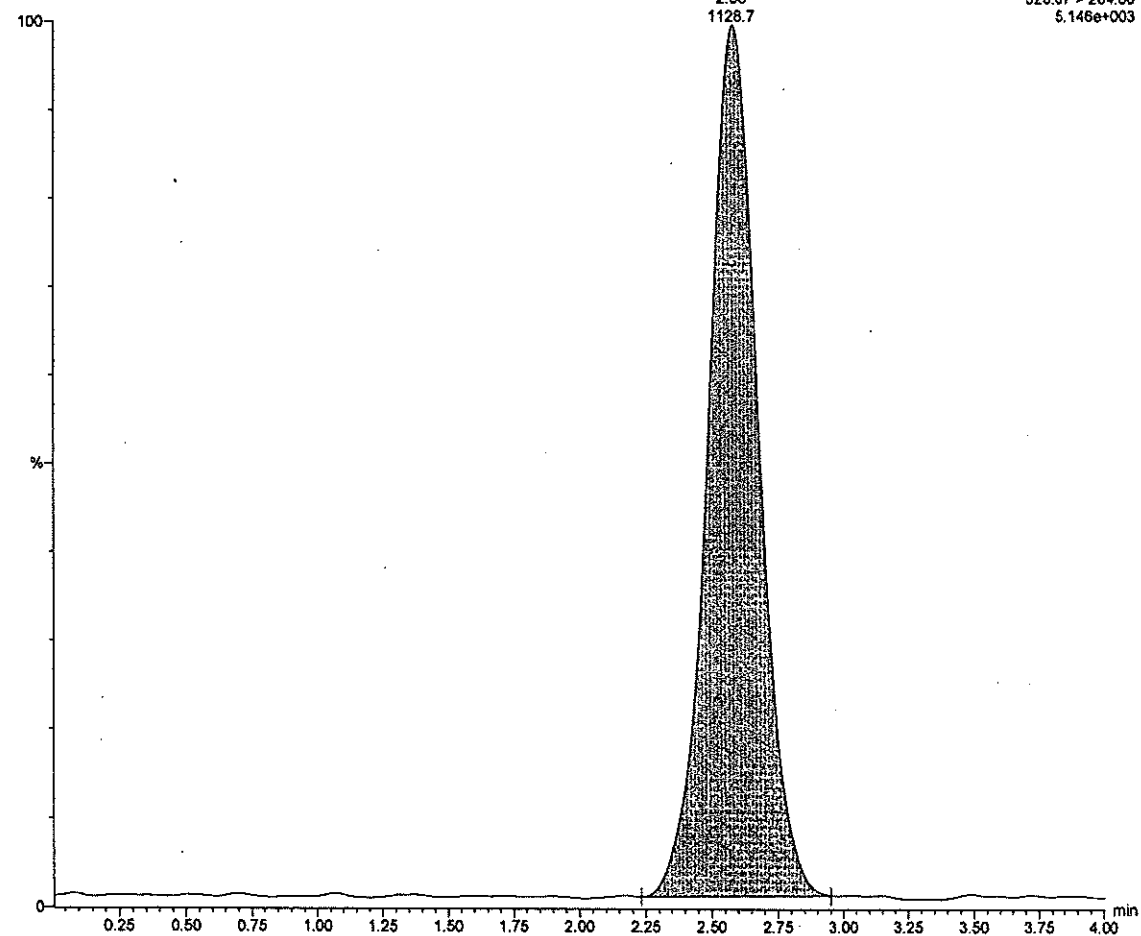
Vial: 1:A,5

User: 志

FRD 903

WH4D01 Smooth(Mn,3x3)

WH4D



#	Name	RT	Area	Height
1	FRD 903	2.583	1128.7	5075

Figure 6 Chromatograms of test water analysis (continued)
day 4, Low concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009

Time: 10:55:18

Description: WL4D

Name: WL4D01

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

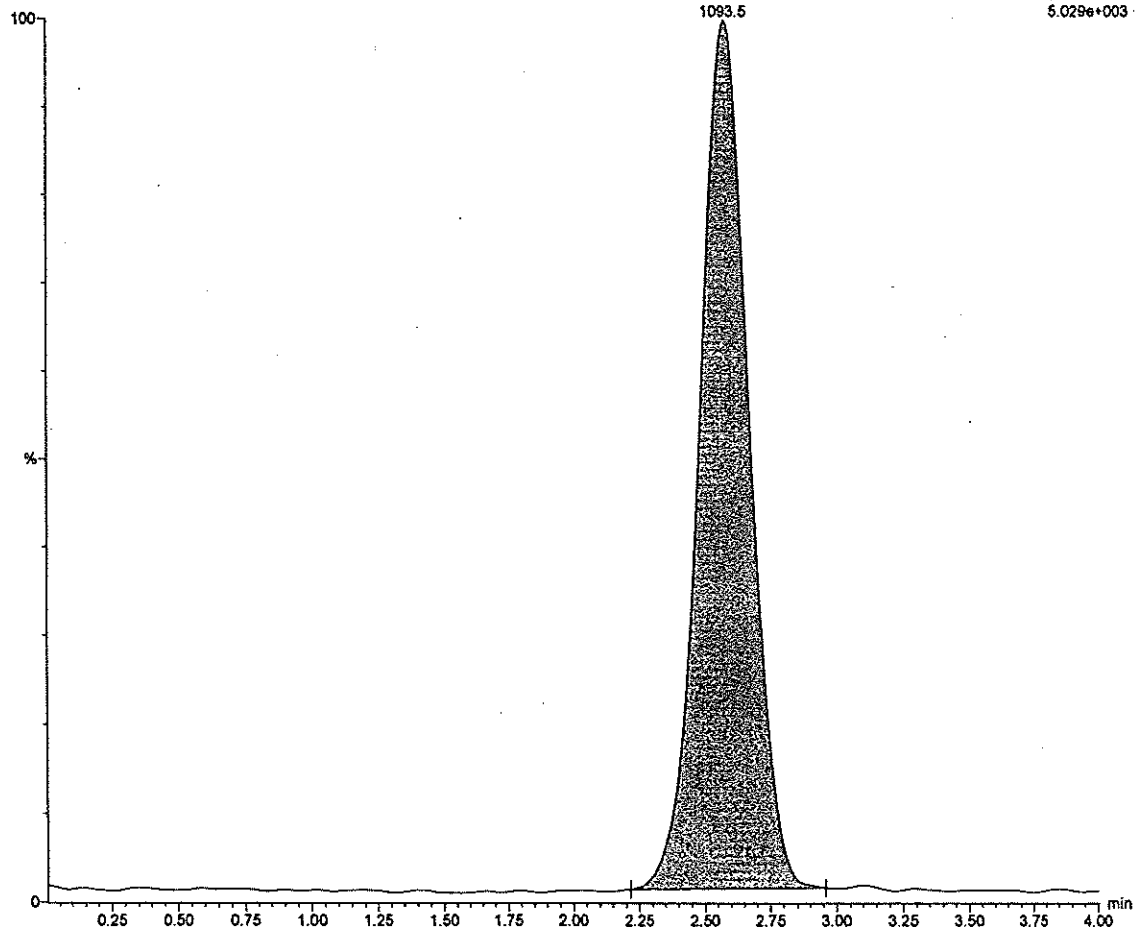
Vial: 1:A,4

User: 志

FRD 903

WL4D01 Smooth(Mn,3x3)

WL4D

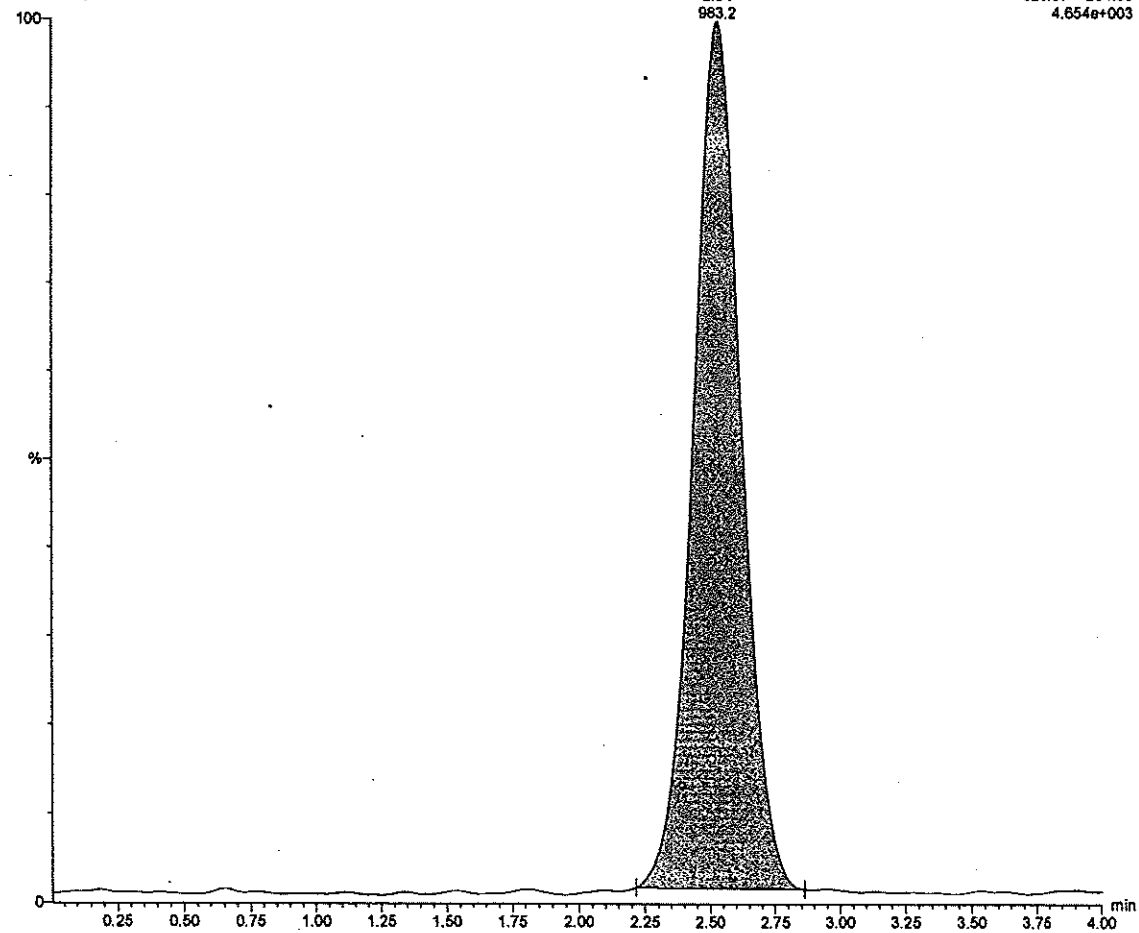


#	Name	RT	Area	Height
1	FRD 903	2.583	1093.5	4950

Figure 7 Chromatograms of test water analysis (continued)

day 7, Std. 0.02 mg/L

Quantify Sample Report
A080558 FRD 903**MassLynx 4.1**

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 23 Apr 2009 11:46:46**Date:** 23-Apr-2009**Time:** 11:23:28**Description:** std 0.02mg/L**Name:** S042303**Inlet Method Name:** C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02**Vial:** 1:A,3**User:** K**FRD 903**S042303 Smooth(Mn,3x3)
std 0.02mg/L

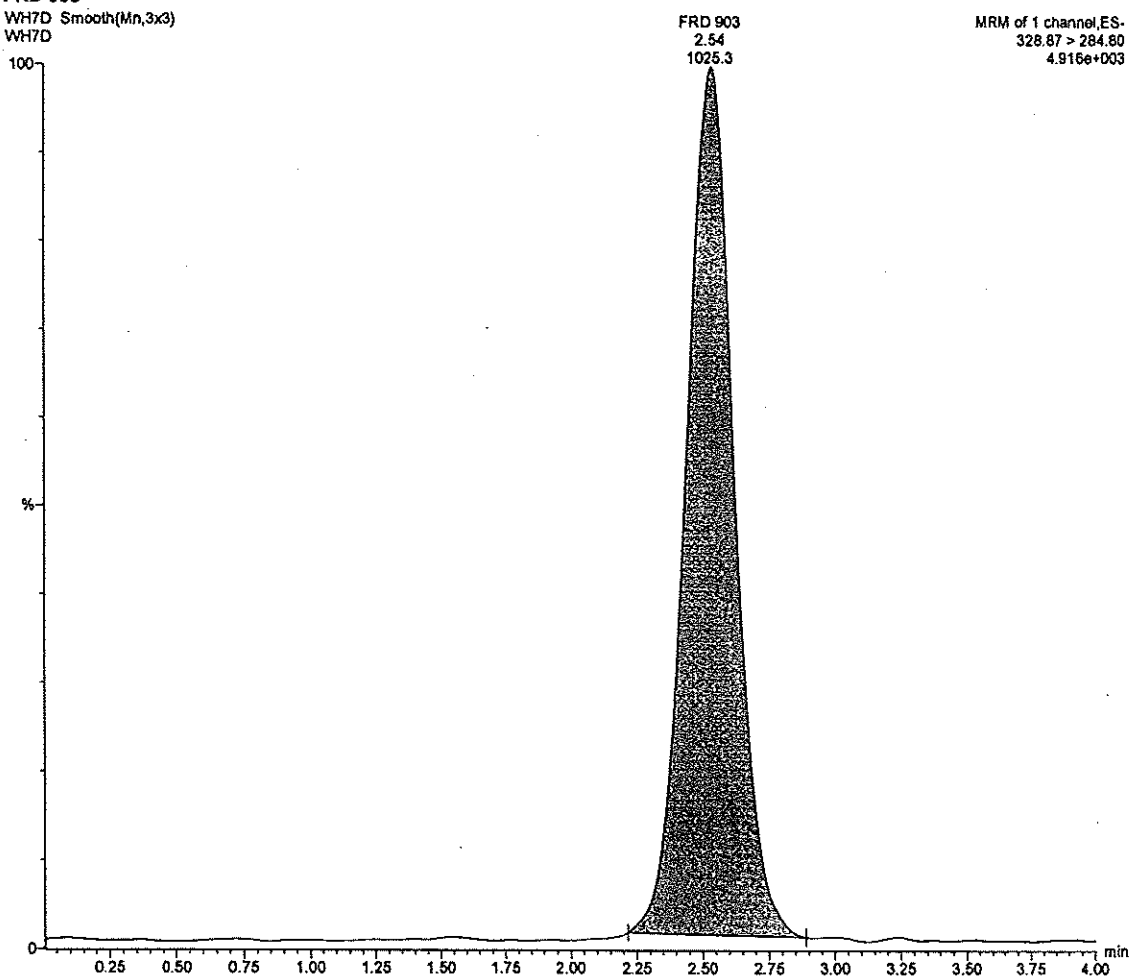
#	Name	RT	Area	Height
1	FRD 903	2.540	983.2	4575

Figure 8 Chromatograms of test water analysis (continued)
day 7, High concentration level

Quantify Sample Report MassLynx 4.1
A080558 FRD 903

Date: 23-Apr-2009
Time: 11:34:39
Description: WH7D
Name: WH7D
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,5
User: 志

FRD 903
WH7D Smooth(Mn,3x3)
WH7D



#	Name	RT	Area	Height
1	FRD 903	2.540	1025.3	4831

Figure 9 Chromatograms of test water analysis (continued)
day 7, Low concentration level

Quantify Sample Report
A080558 FRD 903

MassLynx 4.1

Date: 23-Apr-2009

Time: 11:29:40

Description: WL7D

Name: WL7D

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

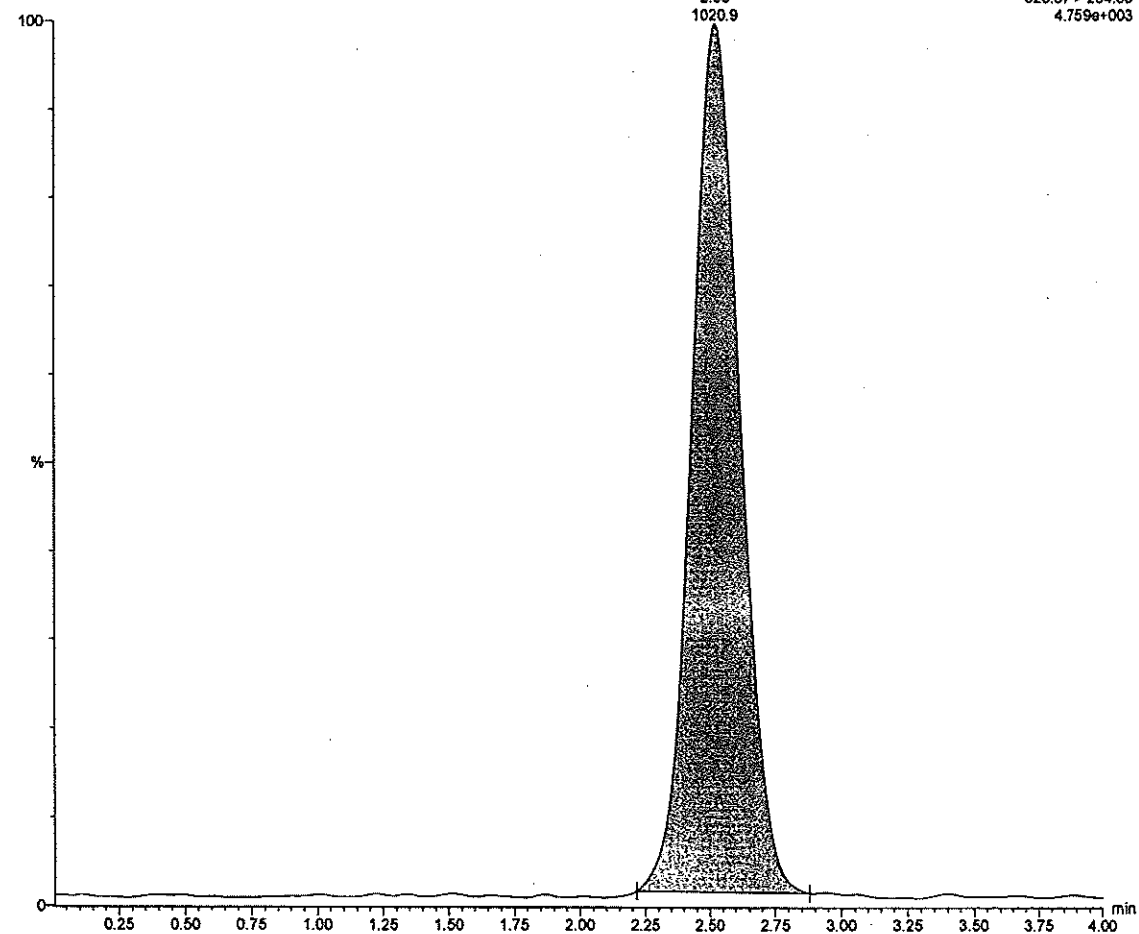
Vial: 1:A,4

User: 志

FRD 903

WL7D Smooth(Mn,3x3)

WL7D



#	Name	RT	Area	Height
1	FRD 903	2.531	1020.9	4680

Figure 10 Chromatograms of test water analysis (continued)
day 14, Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 01 May 2009 17:34:18

Date: 01-May-2009

Time: 17:04:28

Description: std 0.02mg/L

Name: S050103

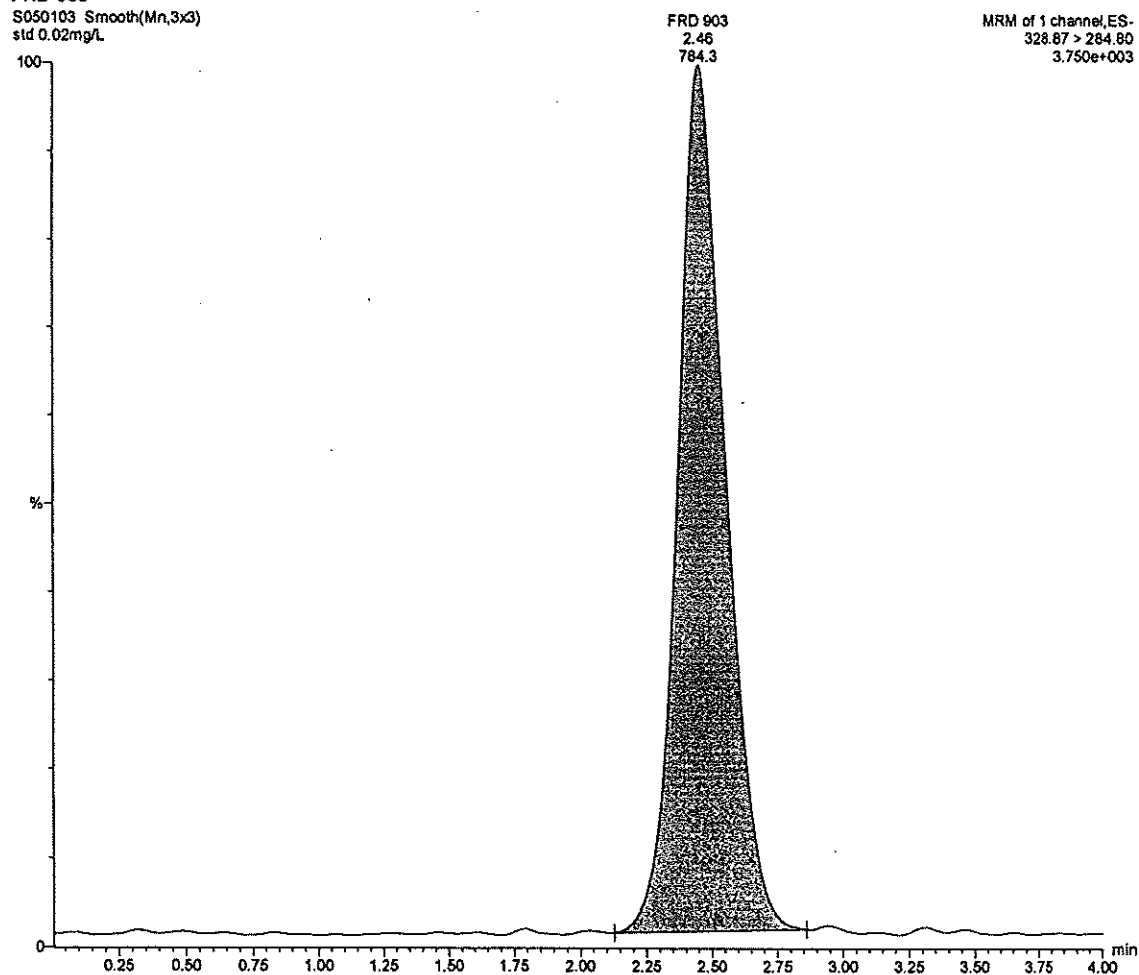
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,B

User: (C)

FRD 903


S050103 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.461	784.3	3680

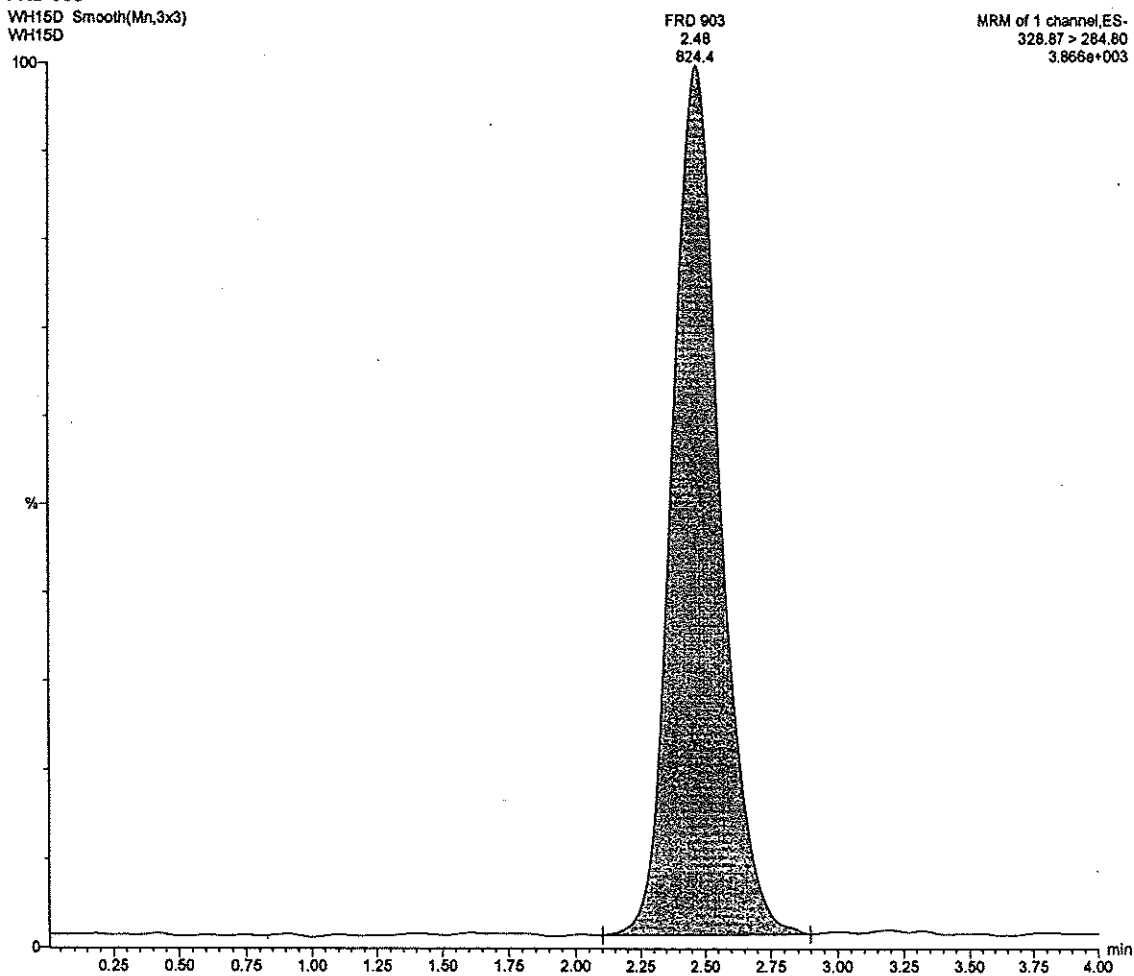
Figure 11 Chromatograms of test water analysis (continued)
day 14, High concentration level

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Date: 01-May-2009
Time: 17:17:10
Description: WH15D
Name: WH15D
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,6
User: 

FRD 903

WH15D Smooth(Mn,3x3)
WH15D



#	Name	RT	Area	Height
1	FRD 903	2.478	824.4	3807

Figure 12 Chromatograms of test water analysis (continued)
day 14, Low concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 01-May-2009

Time: 17:12:11

Description: WL15D

Name: WL15D

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

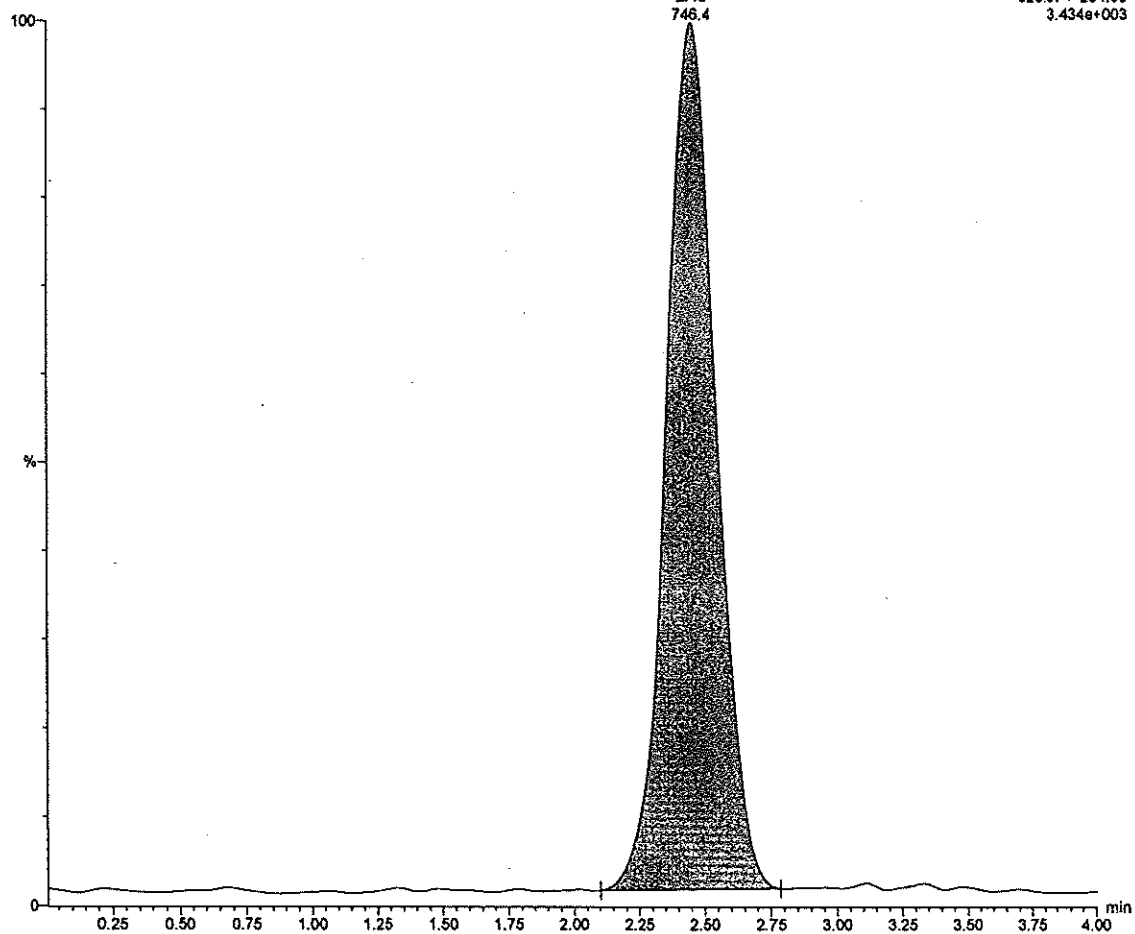
Vial: 1:A,5

User: 

FRD 903

WL15D Smooth(Mn,3x3)

WL15D



MRM of 1 channel, ES-
328.87 > 284.80
3.434e+003

#	Name	RT	Area	Height
1	FRD 903	2.481	746.4	3368

Figure 13 Chromatograms of test water analysis (continued)
day 21, Std. 0.02 mg/L

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 07 May 2009 12:04:41

Date: 07-May-2009

Time: 11:23:32

Description: std 0.02mg/L

Name: S050701

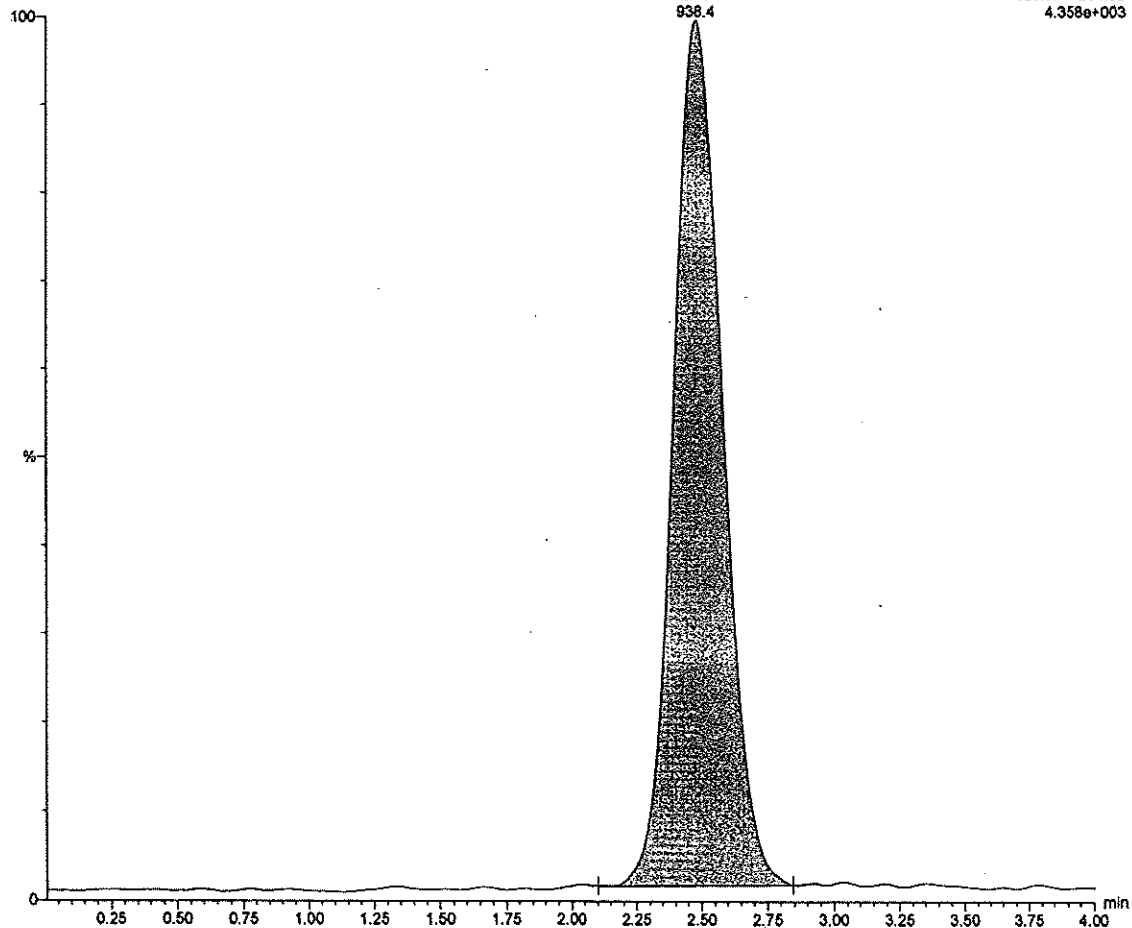
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\UDB\A080560_02

Vial: 1:A,3

User: 志

FRD 903

S050701 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.487	938.4	4284

Figure 14 Chromatograms of test water analysis (continued)
day 21, High concentration level

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009

Time: 11:34:32

Description: WH21D

Name: WH21D

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

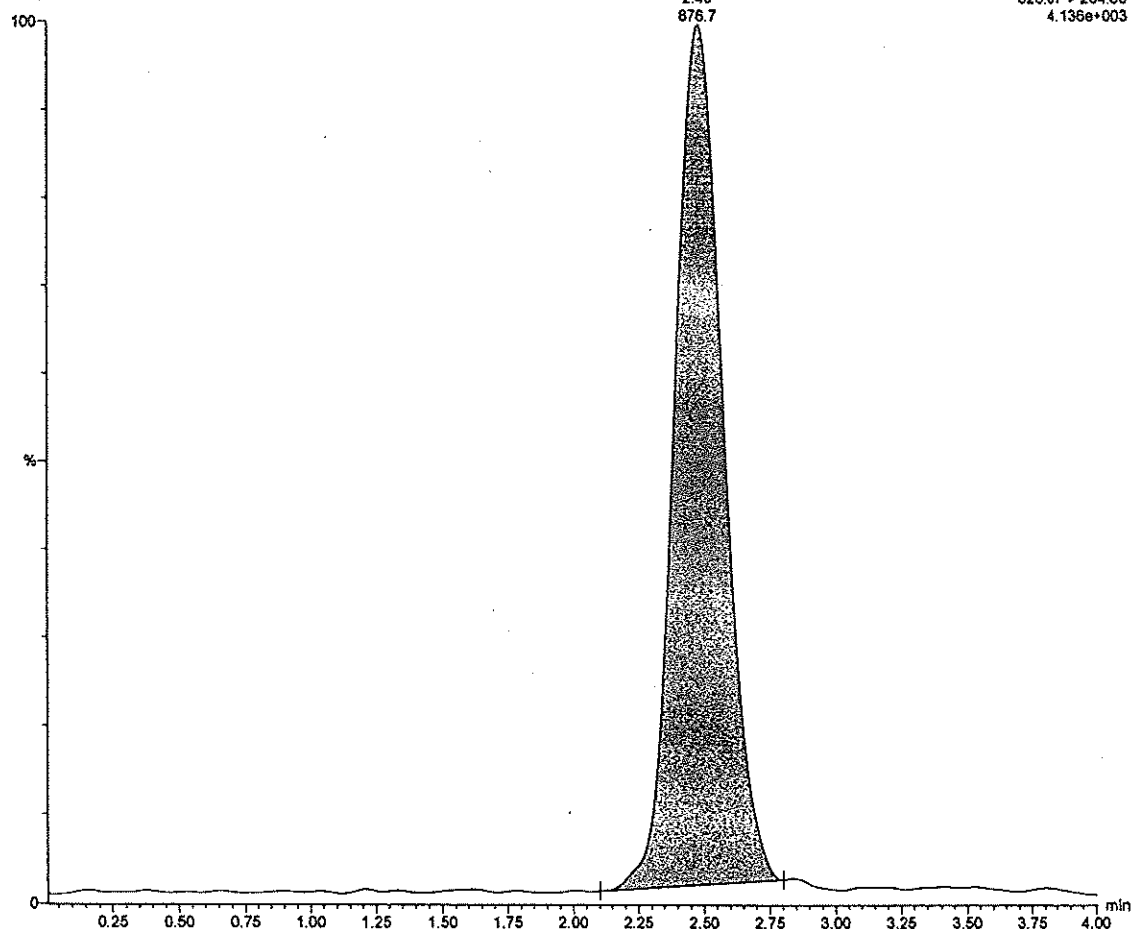
Vial: 1:A,5

User: *JS*

FRD 903

WH21D Smooth(Mn,3x3)

WH21D



#	Name	RT	Area	Height
1	FRD 903	2.487	876.7	4046

Figure 15 Chromatograms of test water analysis (continued)
day 21, Low concentration level

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

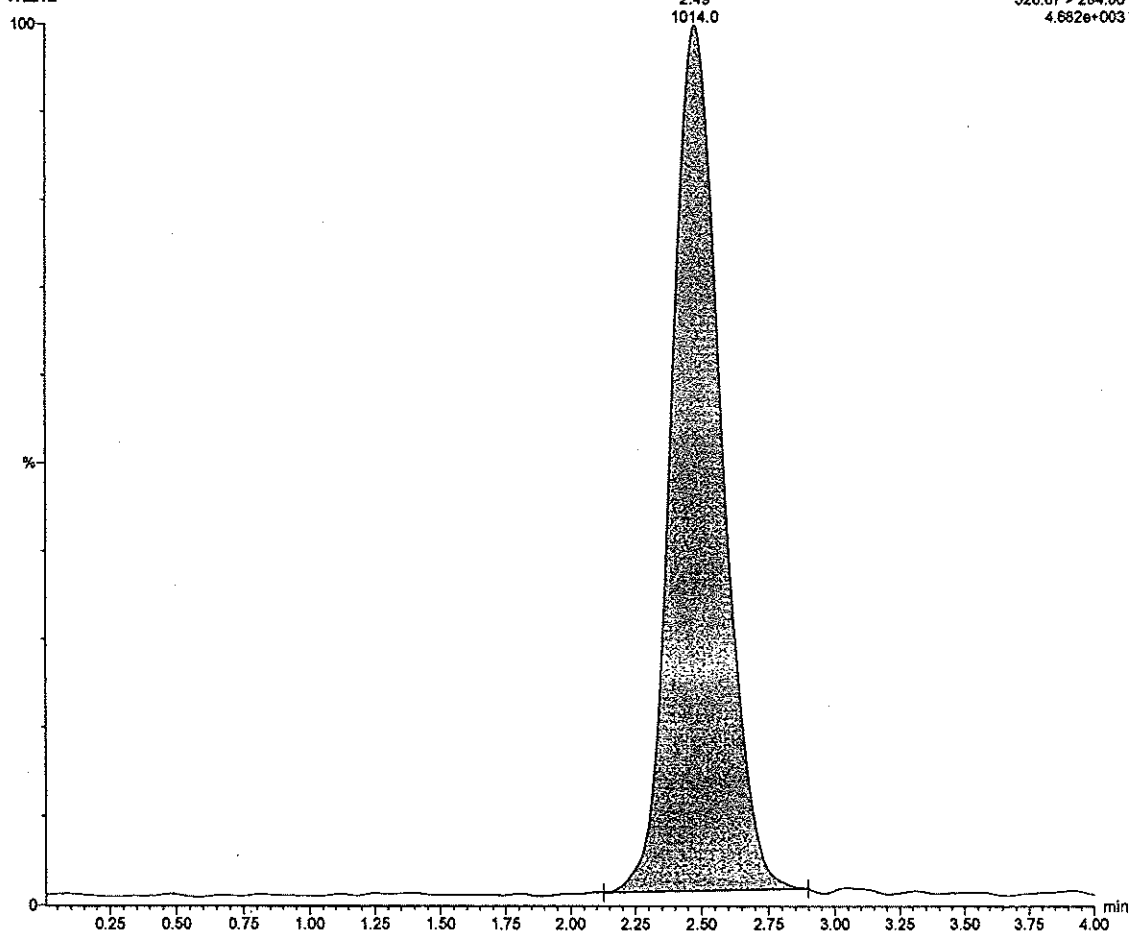
Date: 07-May-2009
Time: 11:29:00
Description: WL21D
Name: WL21D
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDBA080560_02
Vial: 1:A,4
User: 

FRD 903

WL21D Smooth(Mn,3x3)
WL21D

FRD 903
2.49
1014.0

MRM of 1 channel, ES-
328.87 > 284.80
4.682e+003



#	Name	RT	Area	Height
1	FRD 903	2.487	1014.0	4605

Figure 16 Chromatograms of test water analysis (continued)
day 28, Std. 0.02 mg/L

Quantify Sample Report MassLynx 4.1
A080560 FRD903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 14 May 2009 11:27:20

Date: 14-May-2009

Time: 10:56:45

Description: std 0.02mg/L

Name: S051404

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\UDB\A080560_02

Vial: 1:A,3

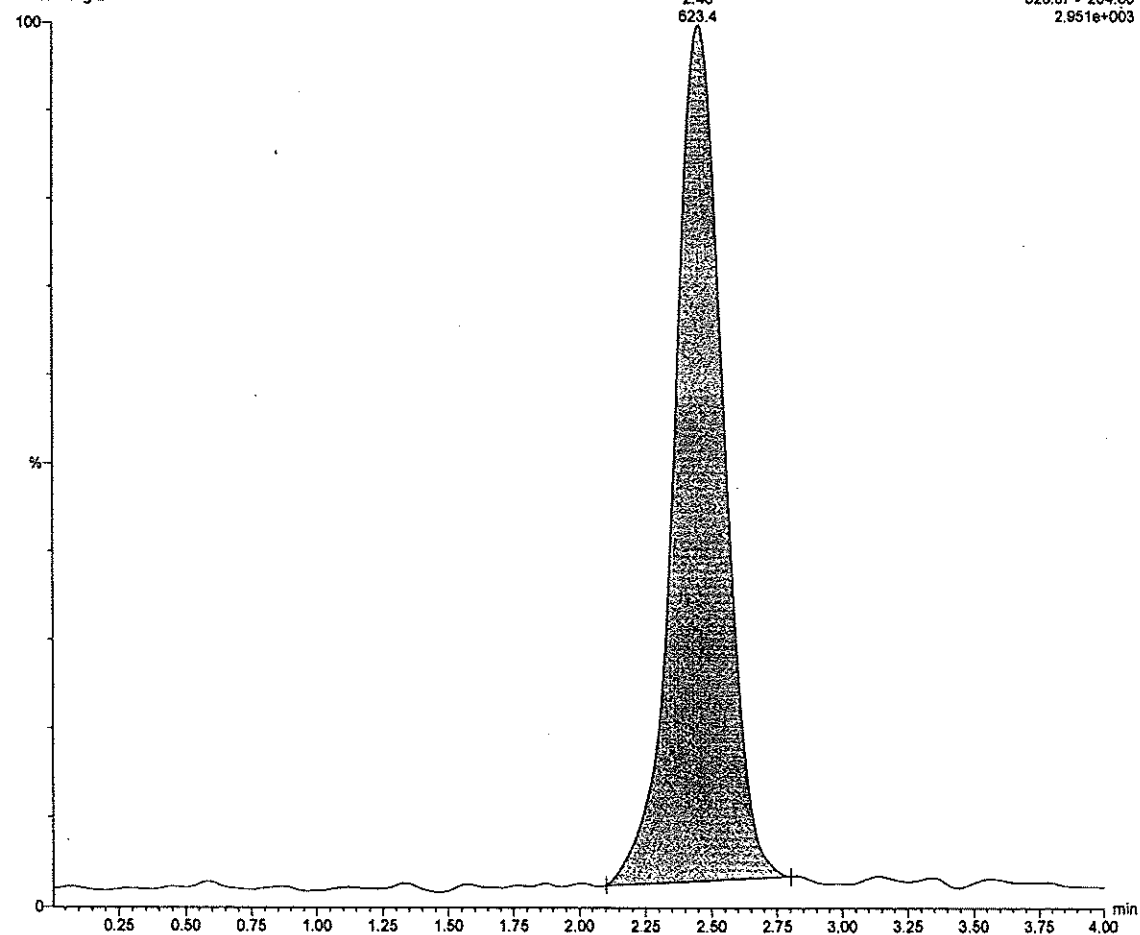
User: *ky*

FRD 903

S051404 Smooth(Mn,3x3)
std 0.02mg/L

FRD 903
2.46
623.4

MRM of 1 channel, ES-
328.87 > 284.80
2.951e+003



#	Name	RT	Area	Height
1	FRD 903	2.461	623.4	2864

Figure 17 Chromatograms of test water analysis (continued)
day 28, High concentration level

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 14-May-2009

Time: 11:08:30

Description: WH28D

Name: WH28D03

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

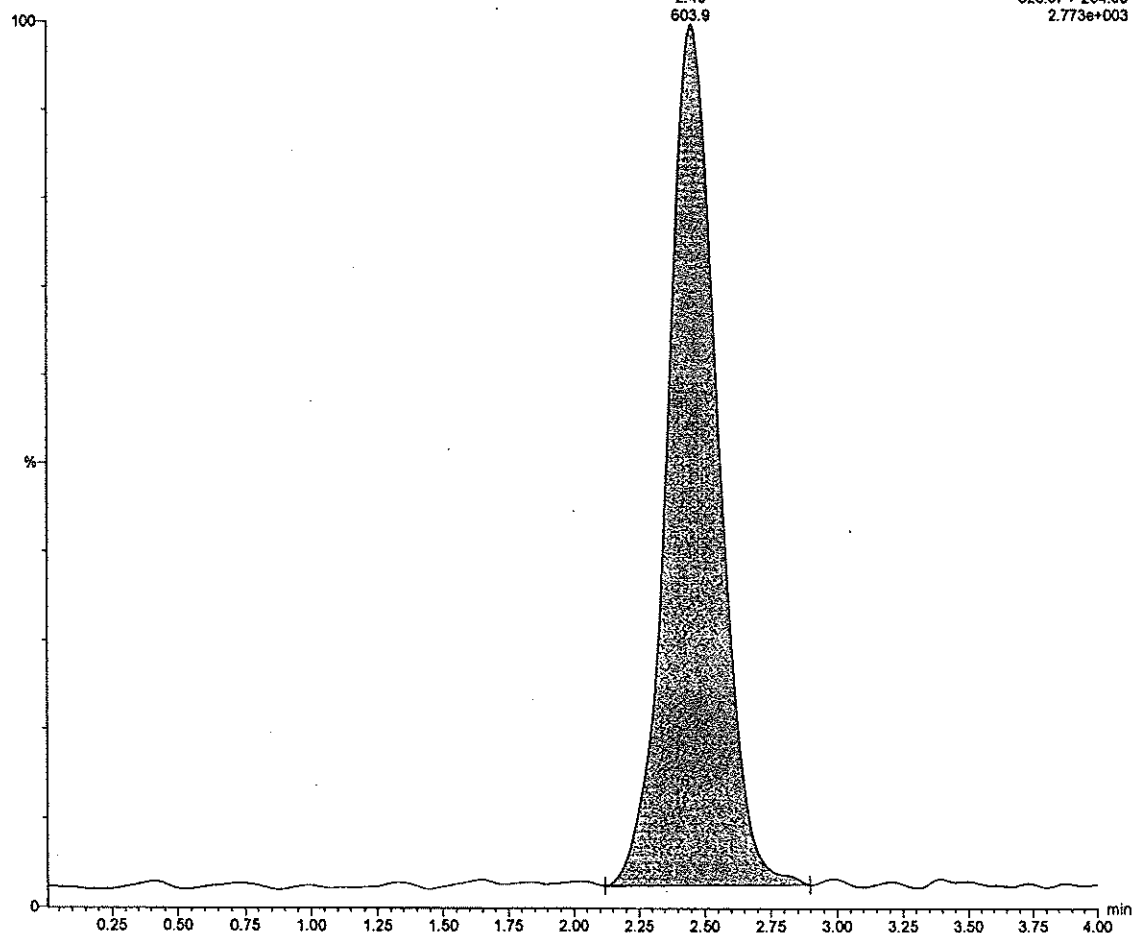
Vial: 1:A,5

User: *xy*

FRD 903

WH28D03 Smooth(Mn,3x3)

WH28D



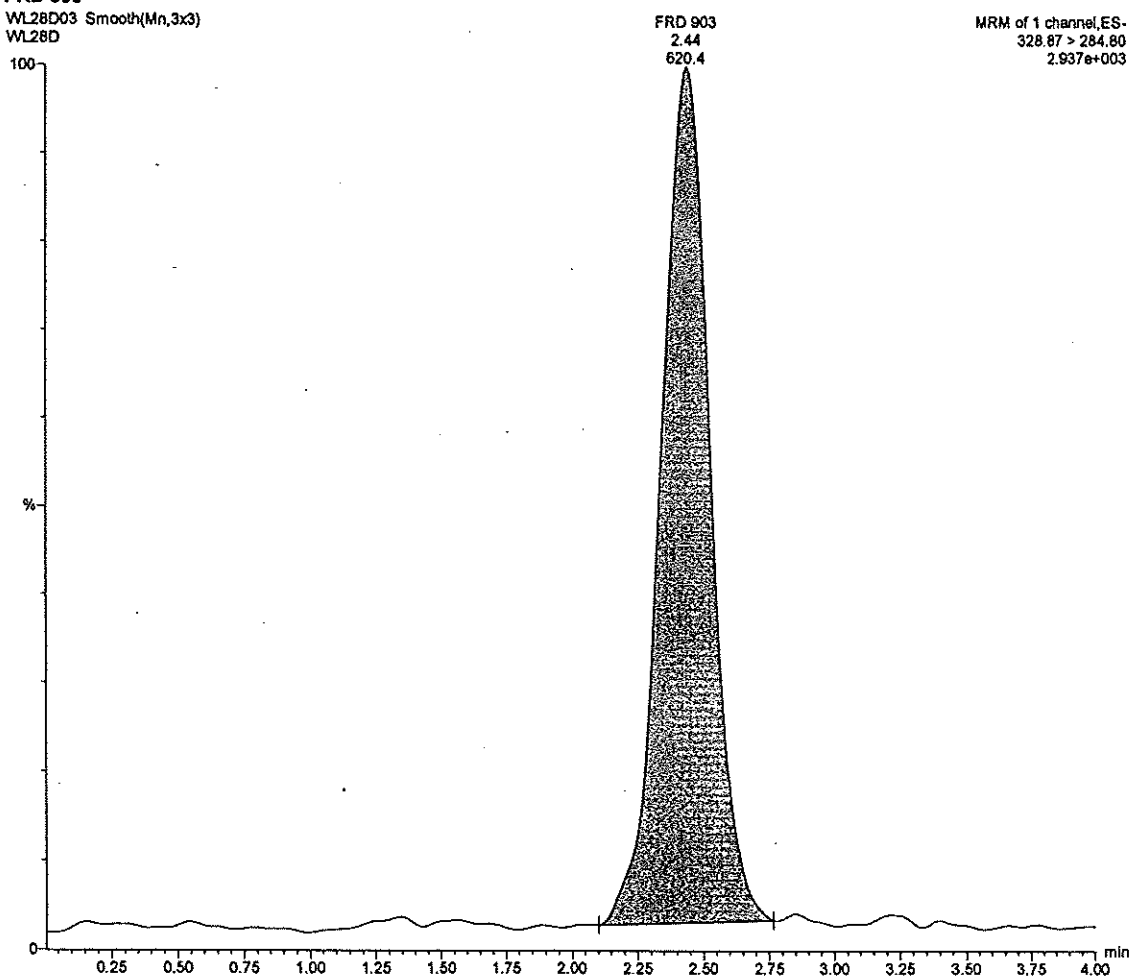
#	Name	RT	Area	Height
1	FRD 903	2.461	603.9	2702

Figure 18 Chromatograms of test water analysis (continued)
day 28, Low concentration level

Quantify Sample Report MassLynx 4.1
A080560 FRD903

Date: 14-May-2009
Time: 11:03:32
Description: WL28D
Name: WL28D03
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:A,4
User: *ky*

FRD 903
WL28D03 Smooth(Mn,3x3)
WL28D



#	Name	RT	Area	Height
1	FRD 903	2.444	620.4	2846

Appendix 6

Chromatograms (Analysis of the test substance in the test fish)

(31 pages)

Figure 1 Chromatograms of test fish analysis
day 4, Std. 0.02 mg/L (High concentration level)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 20 Apr 2009 18:35:32

Date: 20-Apr-2009


Time: 18:01:14

Description: std 0.02mg/L

Name: S042007

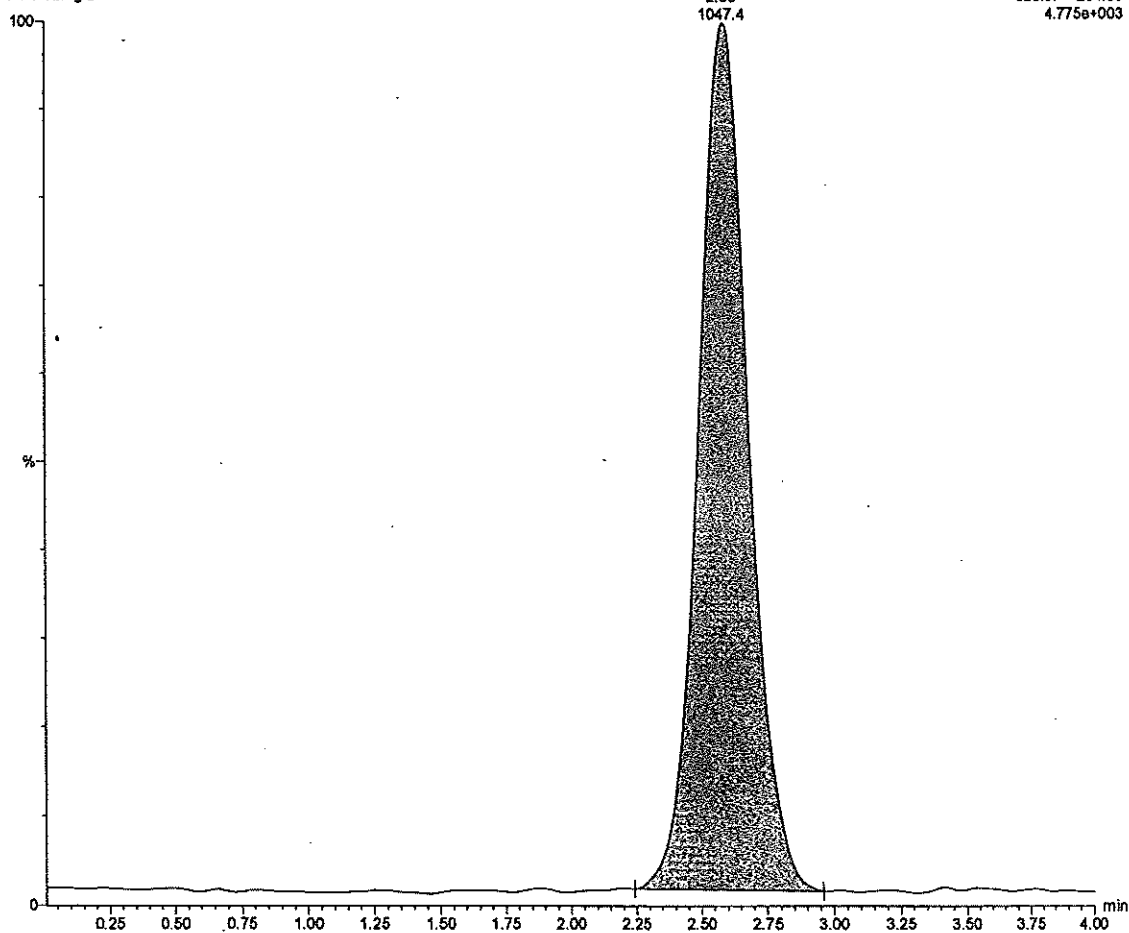
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\BIA080560_02

Vial: 1:A,3

User: 

FRD 903

S042007 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.592	1047.4	4693

Figure 2 Chromatograms of test fish analysis (continued)
day 4, High concentration level, No.1

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009


Time: 18:09:14

Description: 4DFH1

Name: 4DFH103

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

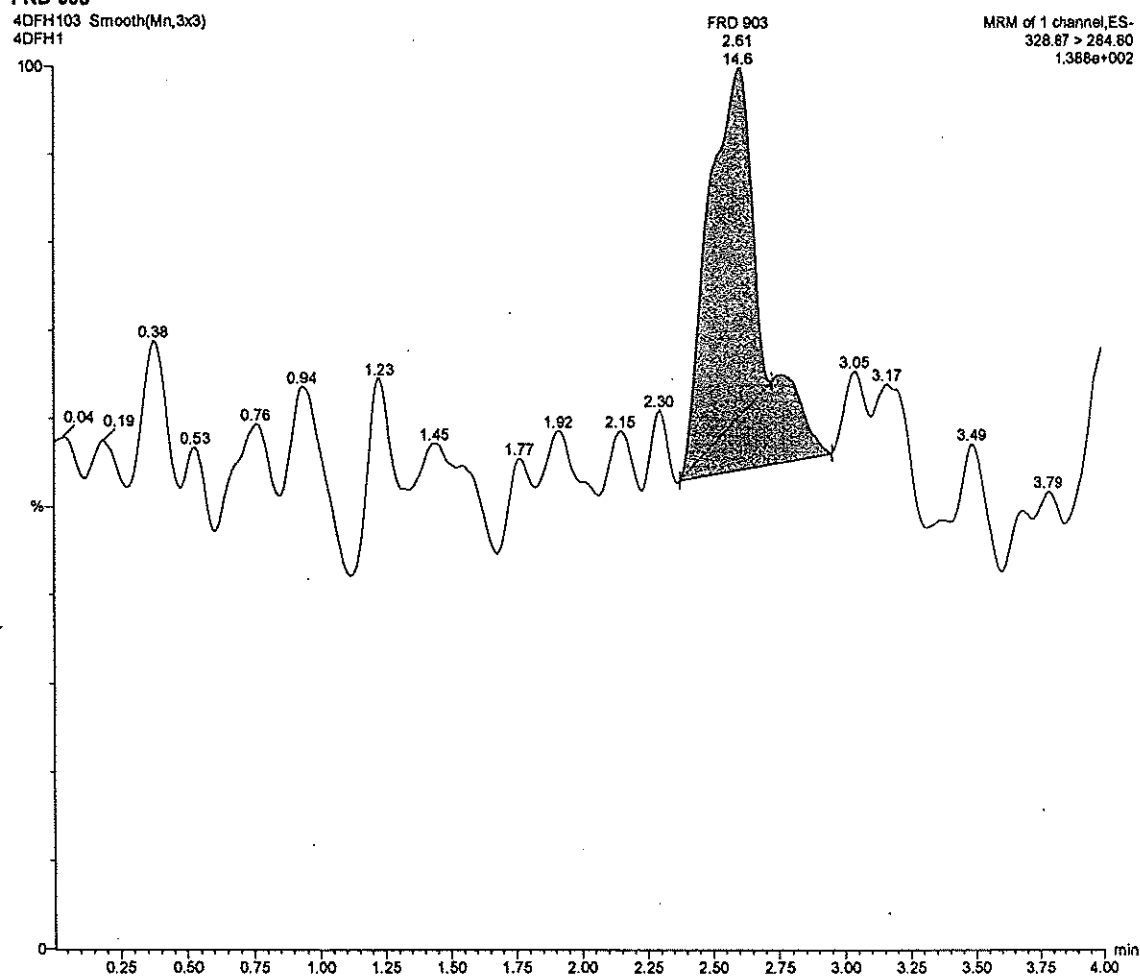
Vial: 1:B,3

User: 

FRD 903

4DFH103 Smooth(Mn,3x3)

4DFH1



#	Name	RT	Area	Height
1	FRD 903	2.610	14.6	63

Figure 3 Chromatograms of test fish analysis (continued)
day 4, High concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009

Time: 18:21:07

Description: 4DFH2

Name: 4DFH203

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,4

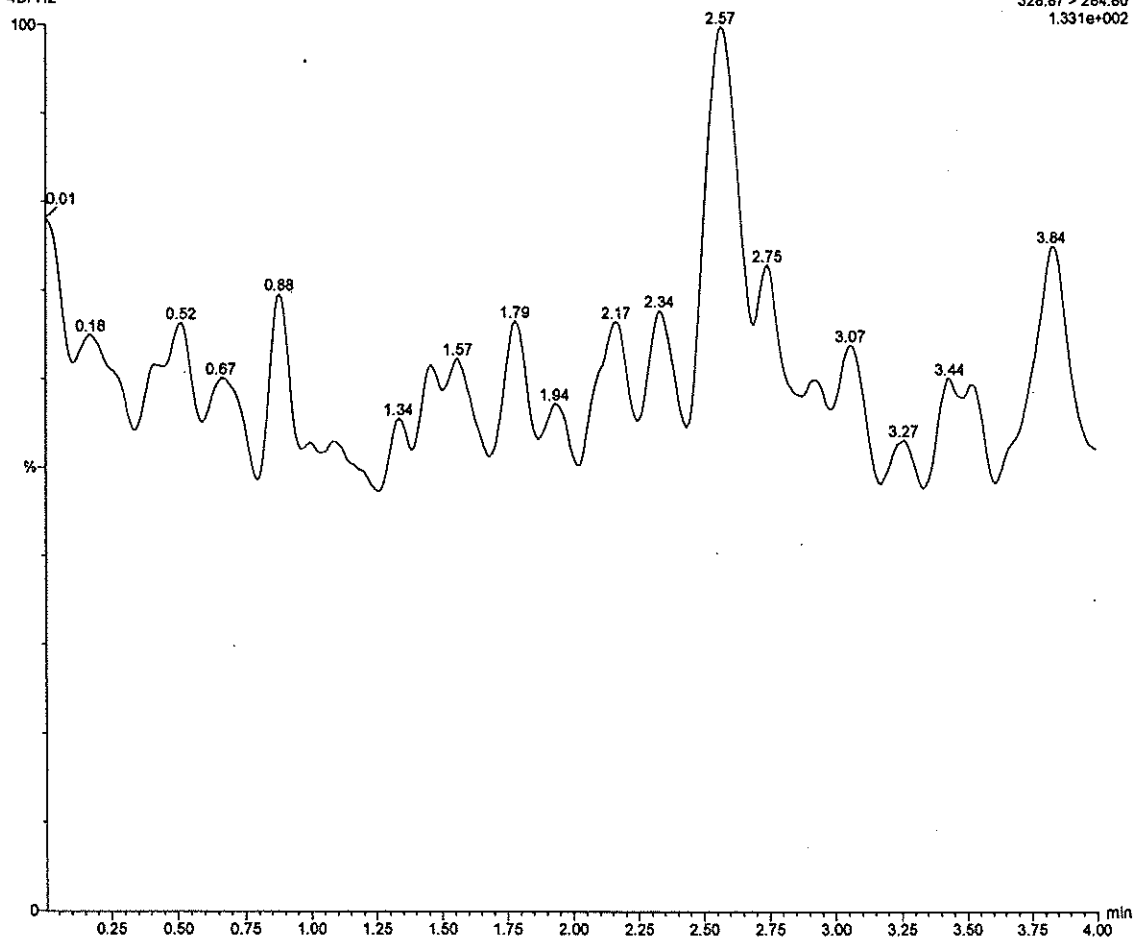
User: 志

FRD 903

4DFH203 Smooth(Mn,3x3)

4DFH2

MRM of 1 channel, ES-
328.87 > 284.80
1.331e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 4 Chromatograms of test fish analysis
day 4, Std. 0.02 mg/L (Low concentration level)

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 20 Apr 2009 18:41:17

Date: 20-Apr-2009


Time: 17:25:04

Description: std 0.02mg/L

Name: S042005

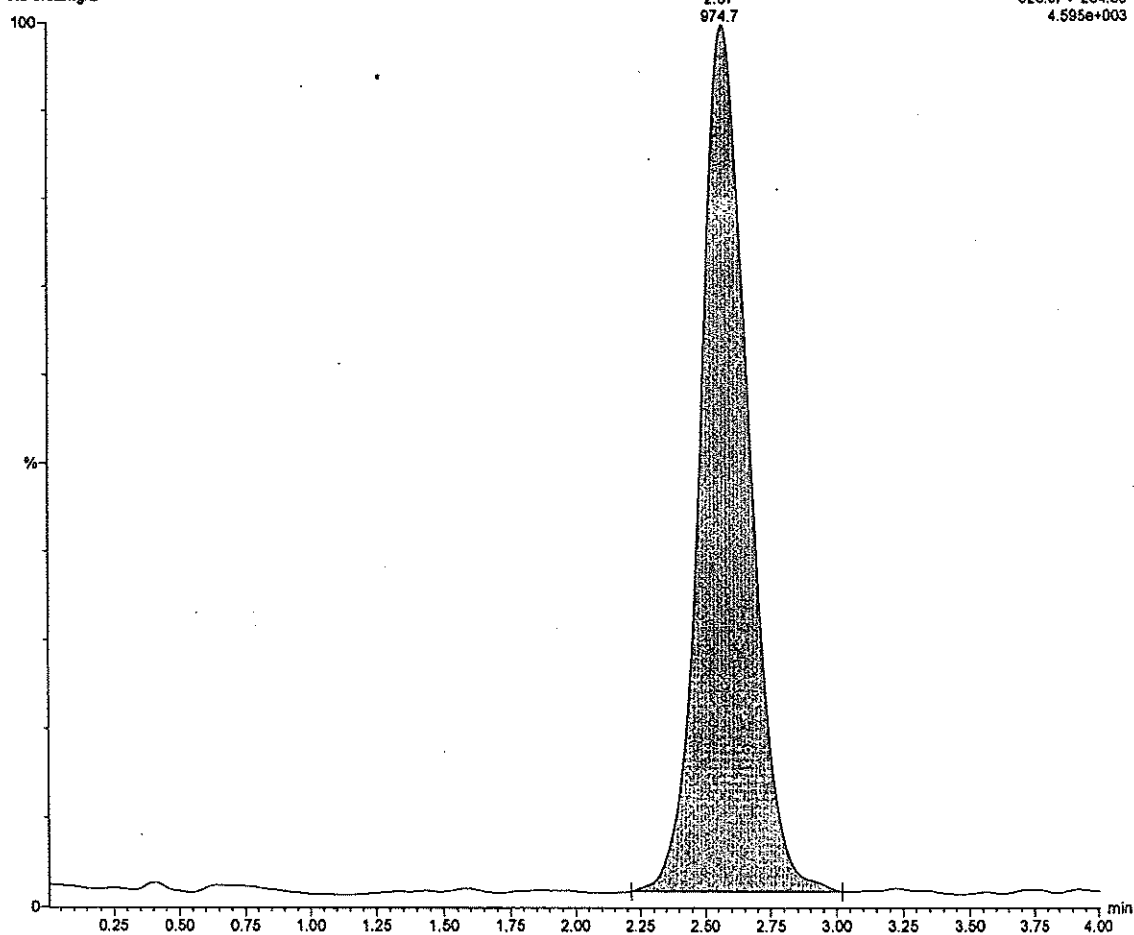
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: 

FRD 903

S042005 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.575	974.7	4514

Figure 5 Chromatograms of test fish analysis (continued)

day 4, Low concentration level, No.1

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009

Time: 17:34:11

Description: 4DFL1

Name: 4DFL102

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,1

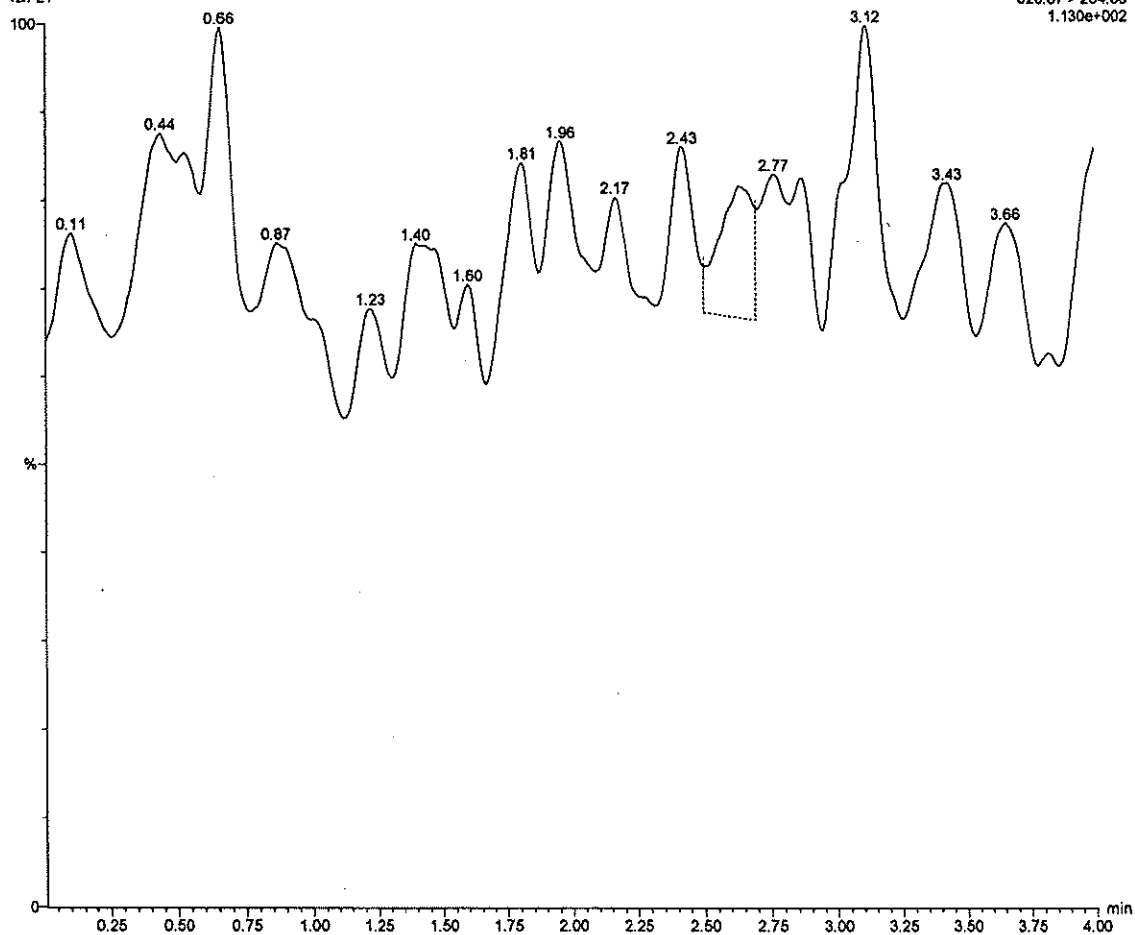
User: 志

FRD 903

4DFL102 Smooth(Mn,3x3)

4DFL1

MRM of 1 channel, ES-
328.87 > 284.80
1.130e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 6 Chromatograms of test fish analysis (continued)

day 4, Low concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 20-Apr-2009

Time: 17:39:09

Description: 4DFL2

Name: 4DFL202

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,2

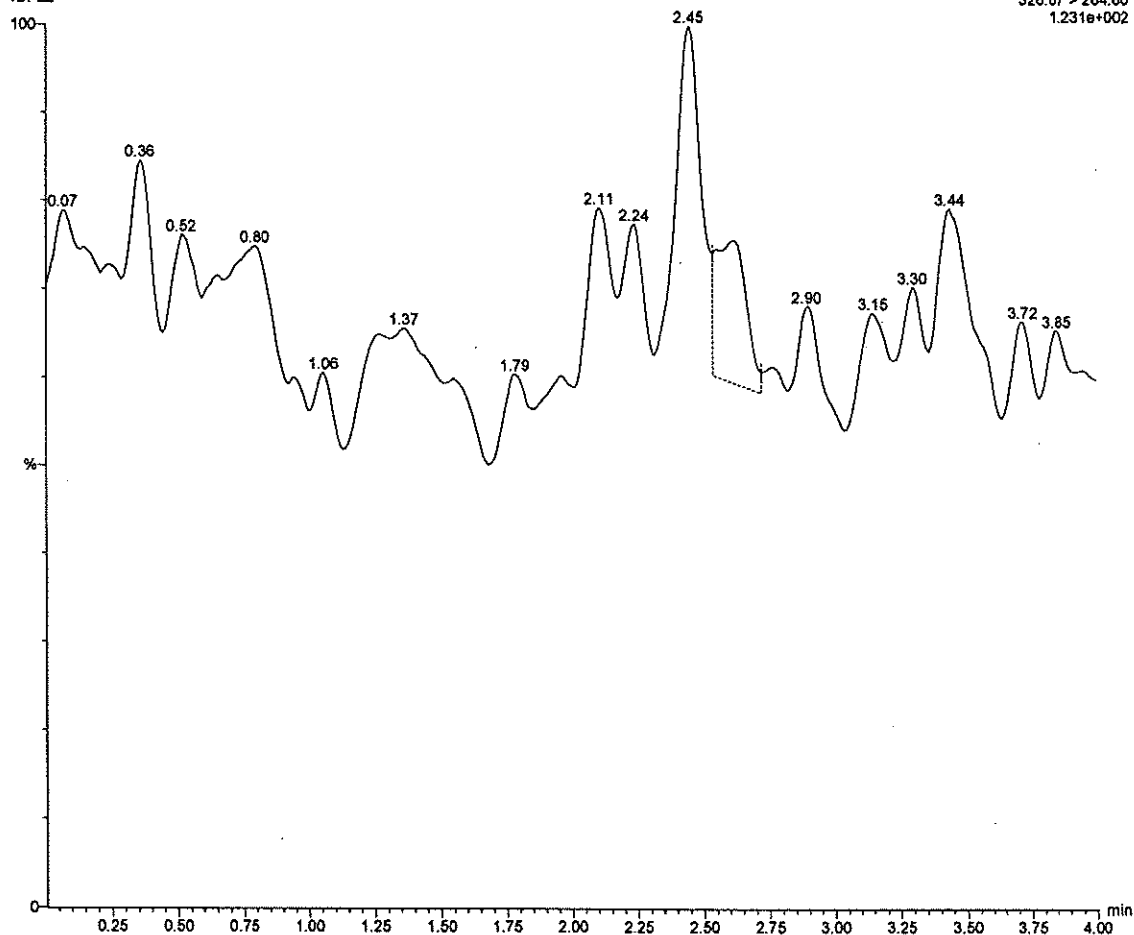
User: 志

FRD 903

4DFL202 Smooth(Mn,3x3)

4DFL2

MRM of 1 channel ES-
328.67 > 284.80
1.231e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 7 Chromatograms of test fish analysis
day 7, Std. 0.02 mg/L (High concentration level)

Quantify Sample Report MassLynx 4.1
A080558 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 23 Apr 2009 17:44:01

Date: 23-Apr-2009

Time: 17:07:45

Description: std 0.02mg/L

Name: S042309

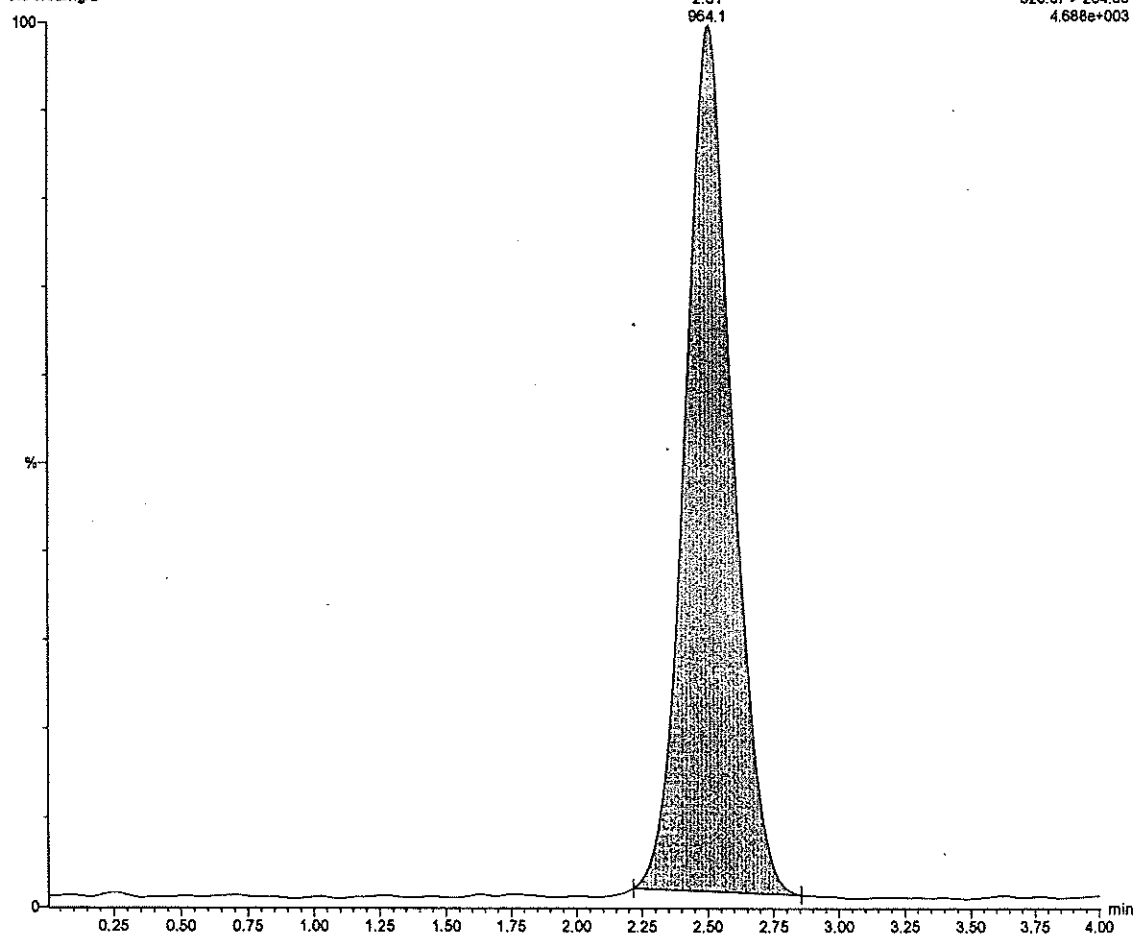
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: *[signature]*

FRD 903

S042309 Smooth(Mn,3x3)
std 0.02mg/L



	Name	RT	Area	Height
1	FRD 903	2.514	964.1	4603

Figure 8 Chromatograms of test fish analysis (continued)
day 7, High concentration level, No.1

Quantify Sample Report
A080558 FRD 903

MassLynx 4.1

Date: 23-Apr-2009

Time: 17:12:43

Description: 7DFH1

Name: 7DFH1

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

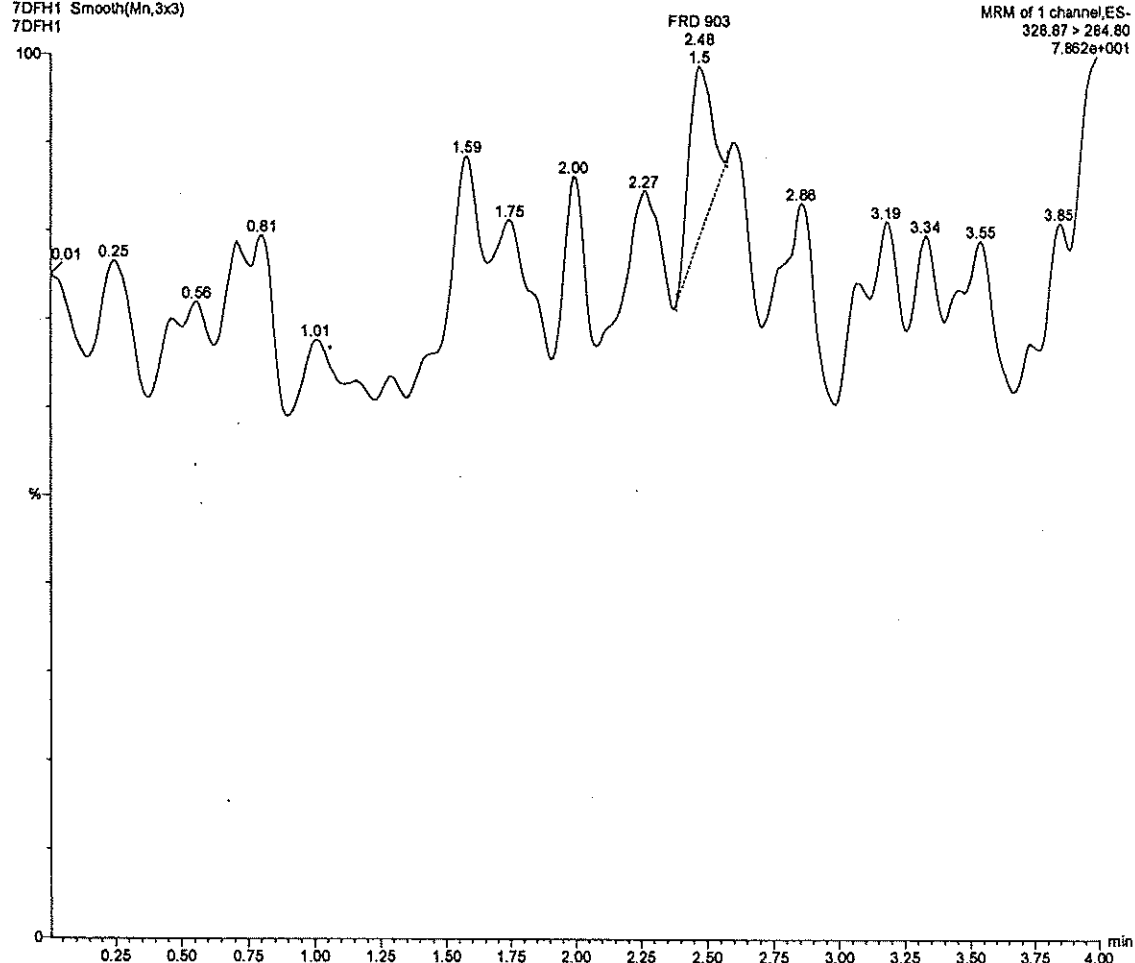
Vial: 1:B,2

User: 志

FRD 903

7DFH1 Smooth(Mn,3x3)

7DFH1



#	Name	RT	Area	Height
1	FRD 903			

Figure 9 Chromatograms of test fish analysis (continued)
day 7, High concentration level, No.2

Quantify Sample Report
A080558 FRD 903

MassLynx 4.1

Date: 23-Apr-2009

Time: 17:18:12

Description: 7DFH2

Name: 7DFH2

Inlet Method Name: C:\MassLynx\biocont\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,3

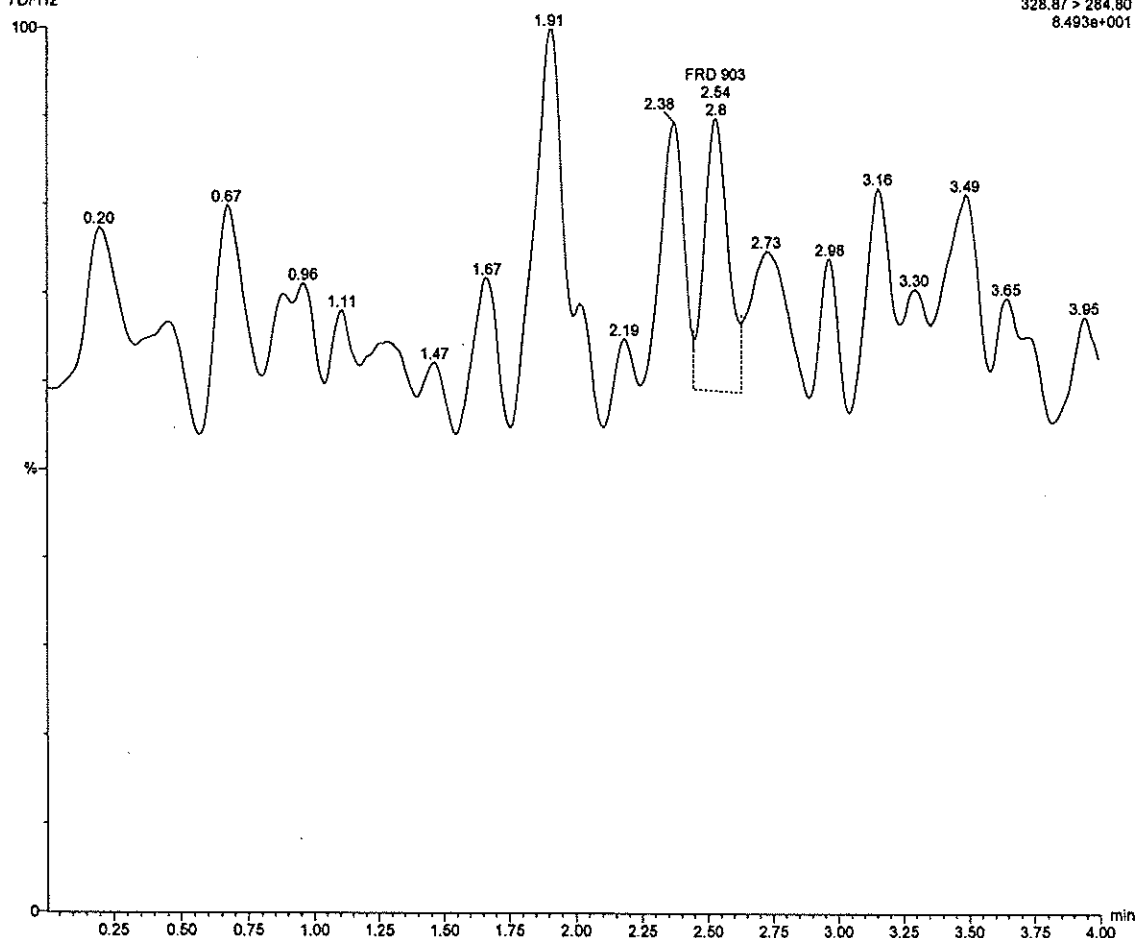
User: *

FRD 903

7DFH2 Smooth(Mn,3x3)

7DFH2

MRM of 1 channel, ES-
328.87 > 284.80
8.493e+001



#	Name	RT	Area	Height
1	FRD 903			

Figure 10 Chromatograms of test fish analysis
day 7, Std. 0.02 mg/L (Low concentration level)

Quantify Sample Report MassLynx 4.1
A080558 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 08 Apr 2009 12:22:29
Calibration: 23 Apr 2009 17:43:17

Date: 23-Apr-2009

Time: 16:36:59

Description: std 0.02mg/L

Name: S042307

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\BIA080560_02

Vial: 1:A,3

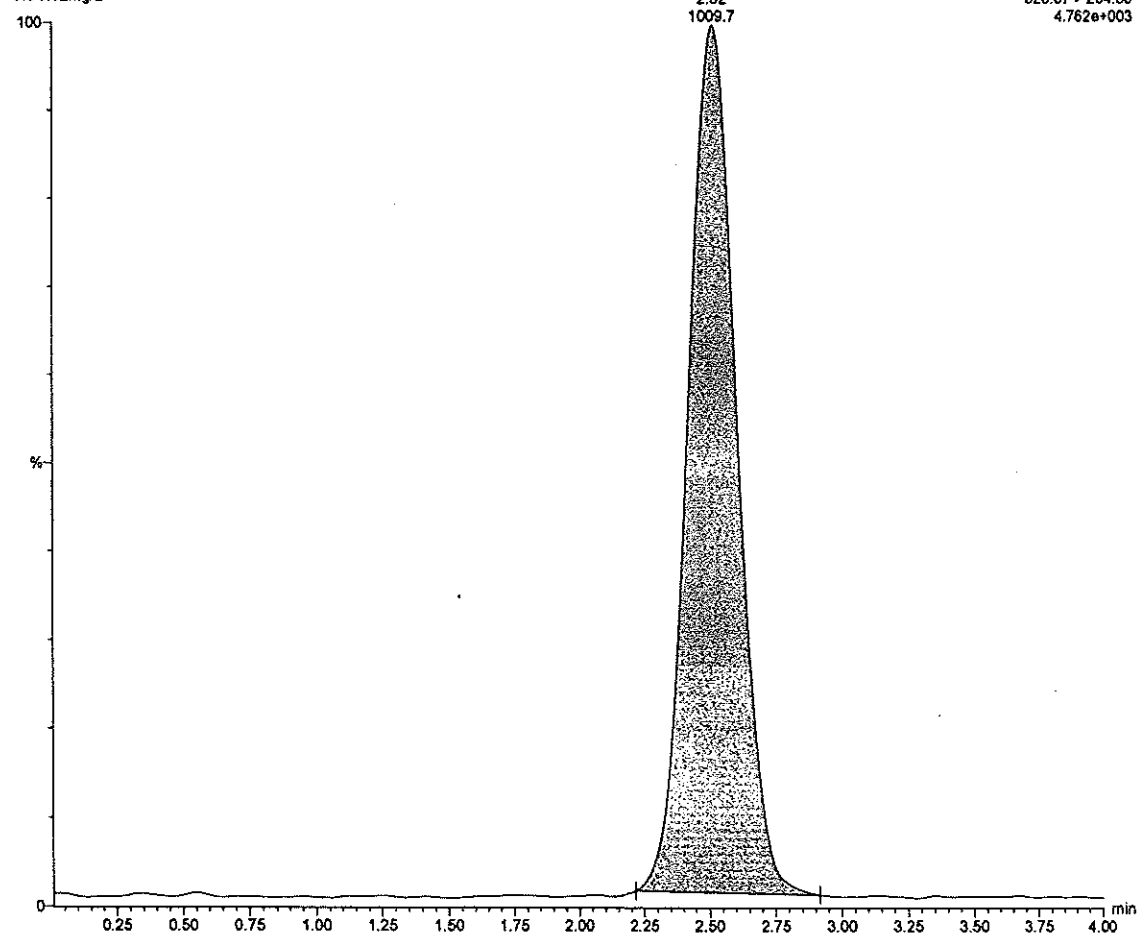
User: *ES*

FRD 903

S042307 Smooth(Mn,3x3)
std 0.02mg/L

FRD 903
2.52
1009.7

MRM of 1 channel, ES-
328.87 > 284.80
4.762e+003



#	Name	RT	Area	Height
1	FRD 903	2.522	1009.7	4683

Figure 11 Chromatograms of test fish analysis (continued)

day 7, Low concentration level, No.1

Quantify Sample Report
A080558 FRD 903

MassLynx 4.1

Date: 23-Apr-2009

Time: 16:41:58

Description: 7DFL1

Name: 7DFL1

Inlet Method Name: C:\MassLynx\biocon\A080560.FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,2

User: 志

FRD 903

7DFL1 Smooth(Mn,3x3)

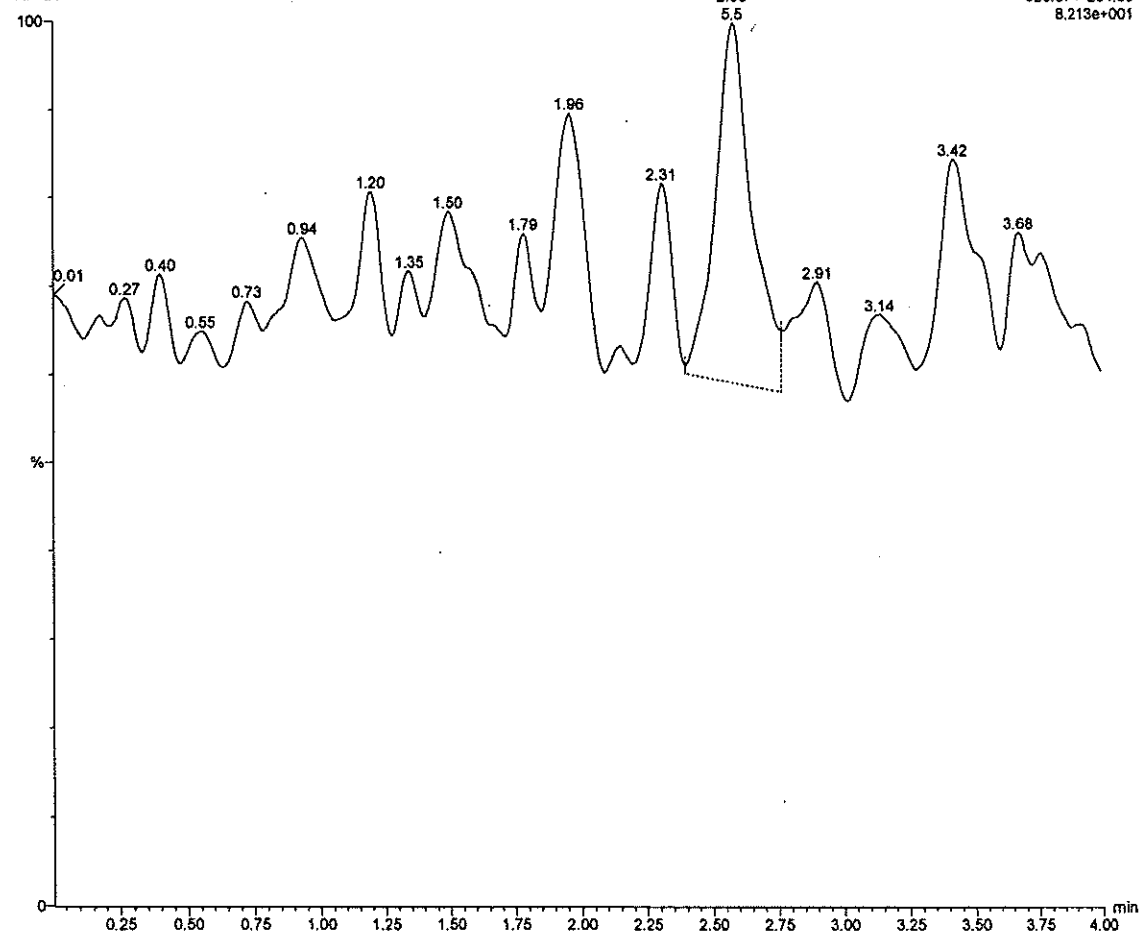
7DFL1

FRD 903

MRM of 1 channel, ES-

328.87 > 284.80

8.213e+001



#	Name	RT	Area	Height
1	FRD 903			

Figure 12 Chromatograms of test fish analysis (continued)
day 7, Low concentration level, No.2

Quantify Sample Report
A080558 FRD 903

MassLynx 4.1

Date: 23-Apr-2009

Time: 16:47:26

Description: 7DFL2

Name: 7DFL2

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,3

User: 志

* 7DF **

* 誤入力 2009.4.23 志

** 誤記 2009.4.23 志

*** 誤記 2009.4.23 志

FRD 903

7DFL2 Smooth(Mn,3x3)

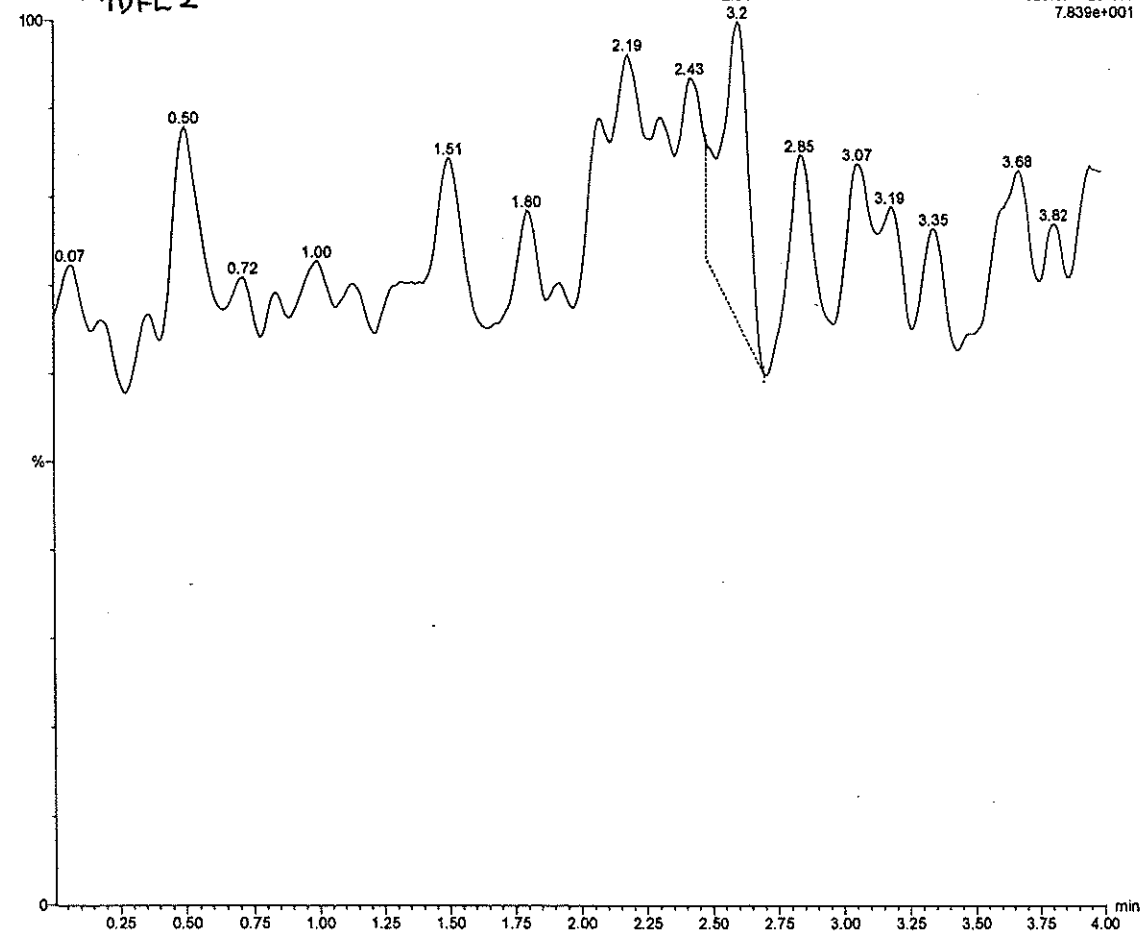
7DFL2

FRD 903

2.61

3.2

MRM of 1 channel, ES-
328.07 > 284.80
7.839e+001



#	Name	RT	Area	Height
1	FRD 903			

Figure 13 Chromatograms of test fish analysis
day 15, Std. 0.02 mg/L (High concentration level)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009

Time: 17:20:04

Description: std 0.02mg/L

Name: S050707

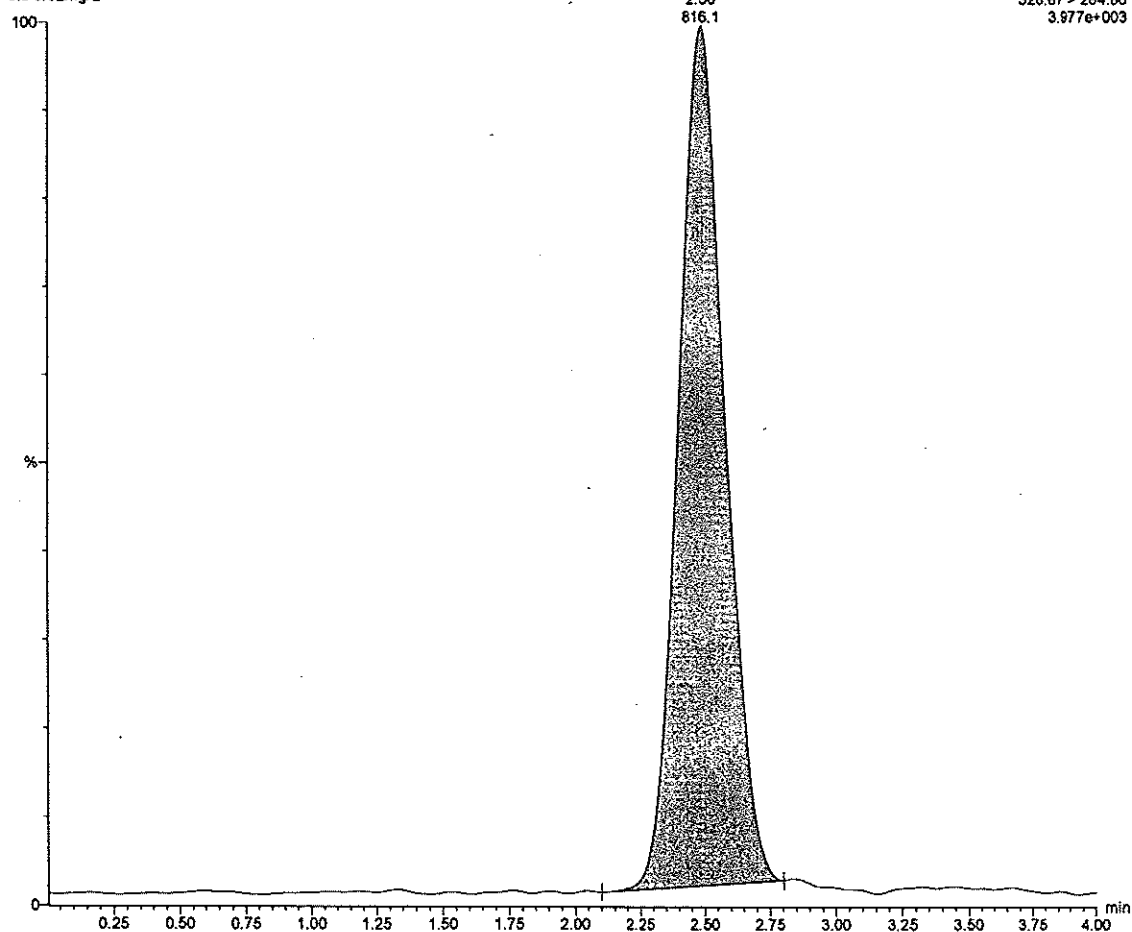
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: *JS*

FRD 903

S050707 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.496	816.1	3883

Figure 14 Chromatograms of test fish analysis (continued)
day 15, High concentration level, No.1

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009


Time: 17:25:35

Description: 15DFH1

Name: 15DFH1

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

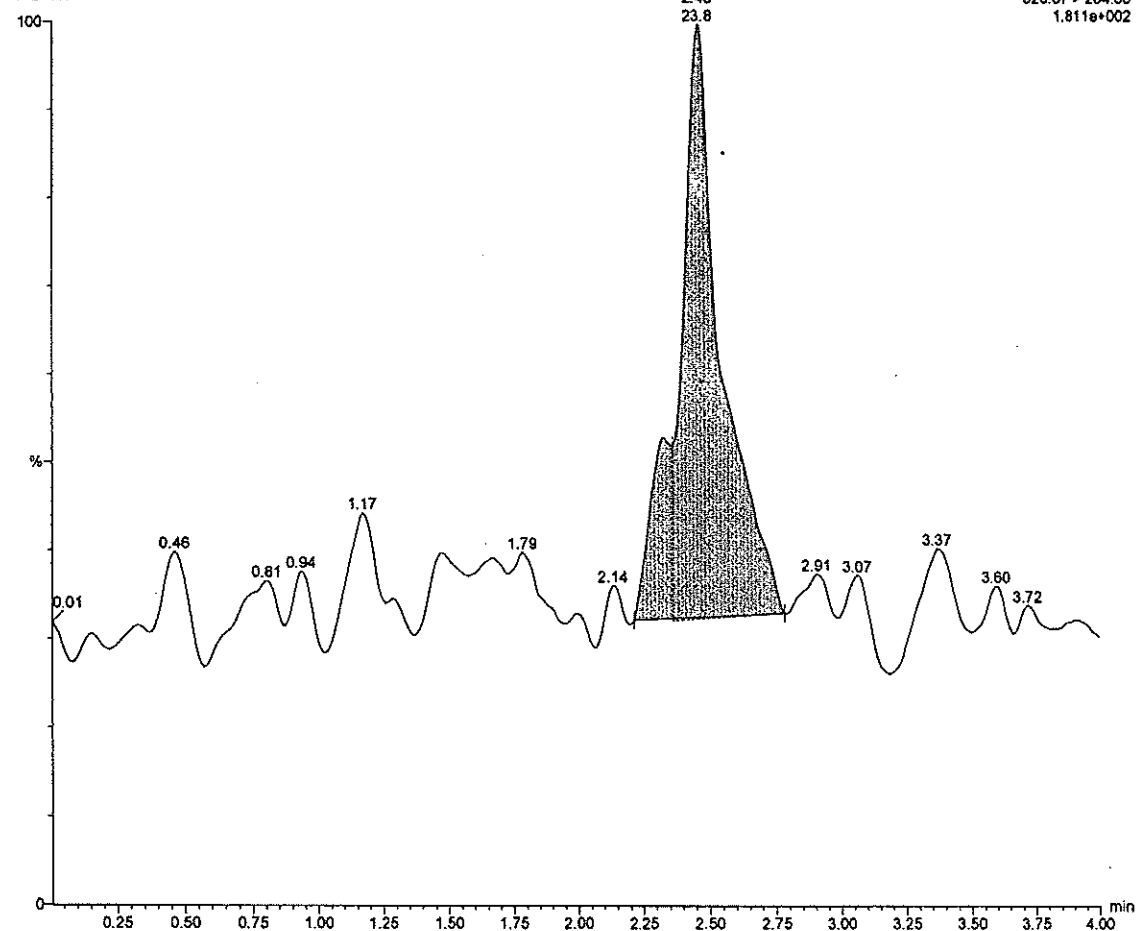
Vial: 1:B,1

User: 

FRD 903

15DFH1 Smooth(Mn,3x3)

15DFH1



#	Name	RT	Area	Height
1	FRD 903	2.461	23.8	122

Figure 15 Chromatograms of test fish analysis (continued)

day 15, High concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009

Time: 17:31:07

Description: 15DFH2

Name: 15DFH2

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

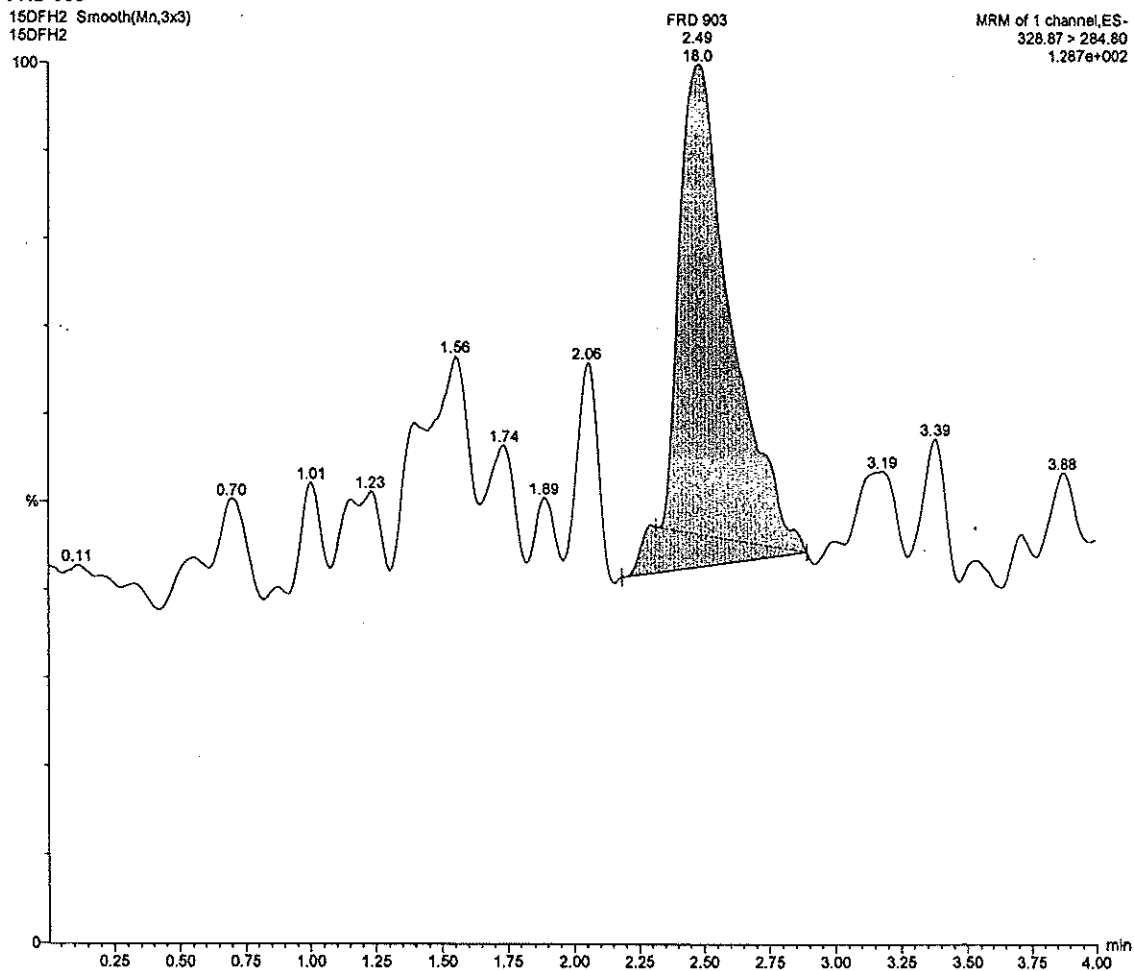
Vial: 1:B,2

User: *JS*

FRD 903

15DFH2 Smooth(Mn,3x3)

15DFH2



#	Name	RT	Area	Height
1	FRD 903	2.487	18.0	74

Figure 16 Chromatograms of test fish analysis
day 15, Std. 0.02 mg/L (Low concentration level)

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 07 May 2009 19:16:57

Date: 07-May-2009

Time: 17:02:18

Description: std 0.02mg/L

Name: S050706

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

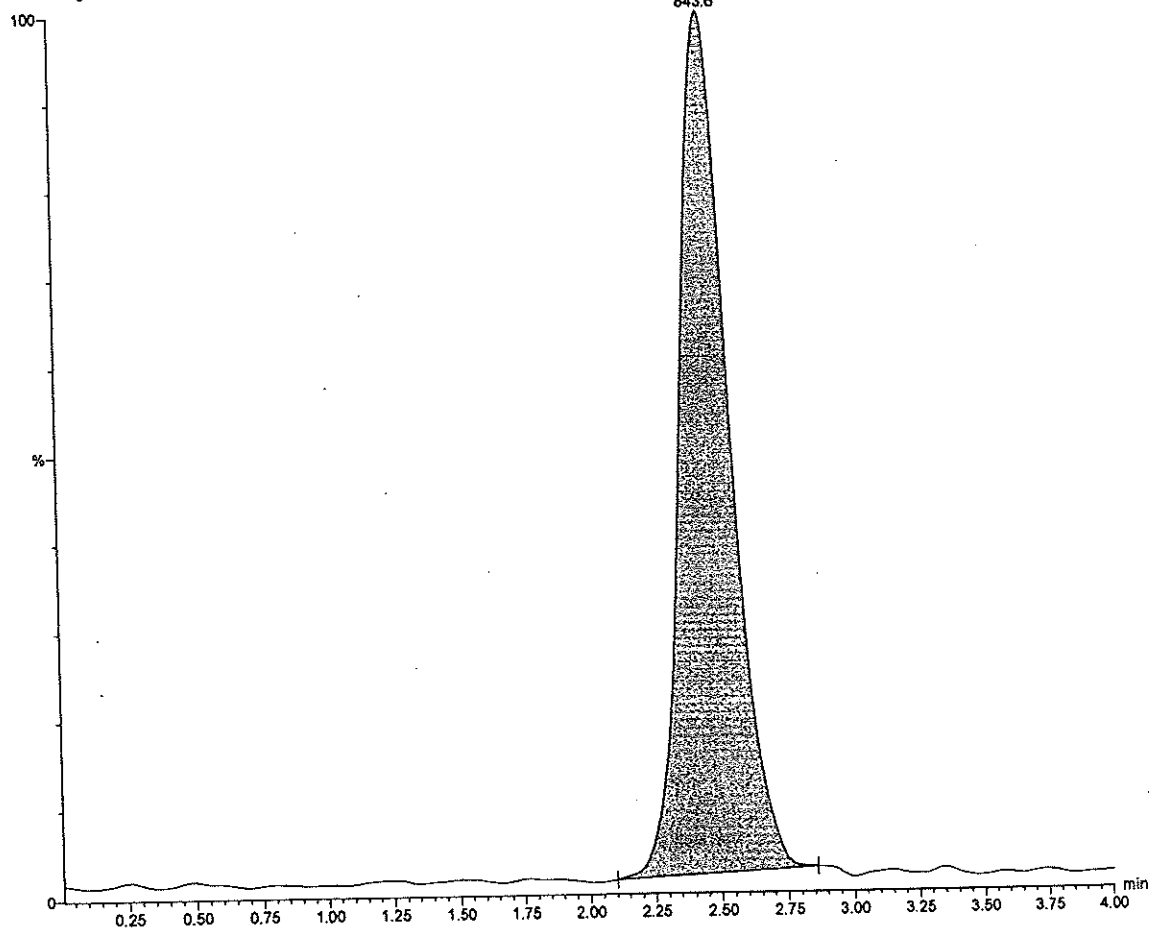
User: *KS*

FRD 903

S050706 Smooth(Mn,3x3)
std 0.02mg/L

FRD 903
2.47
843.6

MRM of 1 channel, ES-
328.87 > 284.80
4.005e+003



#	Name	RT	Area	Height
1	FRD 903	2.470	843.6	3921

Figure 17 Chromatograms of test fish analysis (continued)
day 15, Low concentration level, No.1

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009

Time: 17:09:00

Description: 15DFL1

Name: 15DFL1

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,6

User: *[Signature]*

FRD 903

15DFL1 Smooth(Mn,3x3)

15DFL1

FRD 903

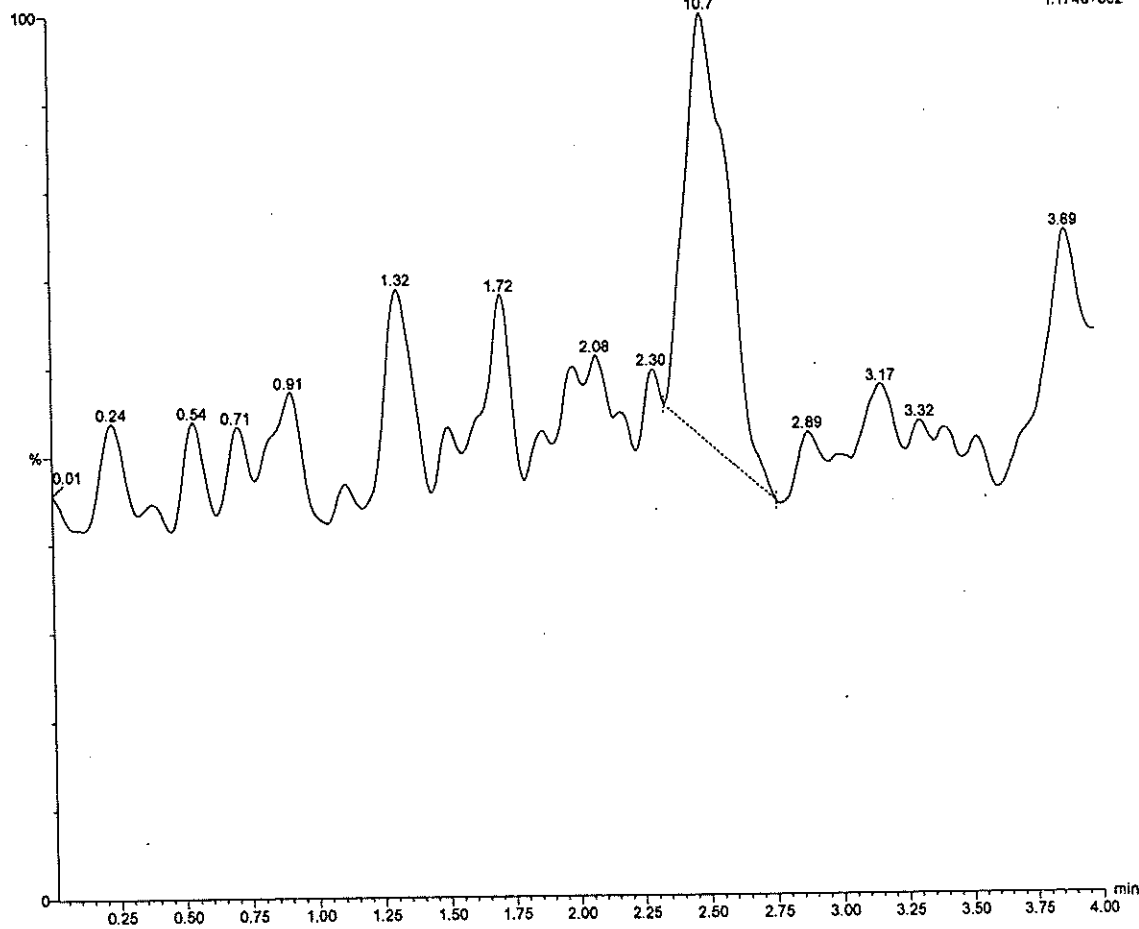
2.50

10.7

MRM of 1 channel ES-

328.87 > 284.80

1.174e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 18 Chromatograms of test fish analysis (continued)
day 15, Low concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 07-May-2009

Time: 17:14:33

Description: 15DFL2

Name: 15DFL2

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,7

User: *sz*

FRD 903

15DFL2 Smooth(Mn,3x3)

15DFL2

FRD 903

2.32

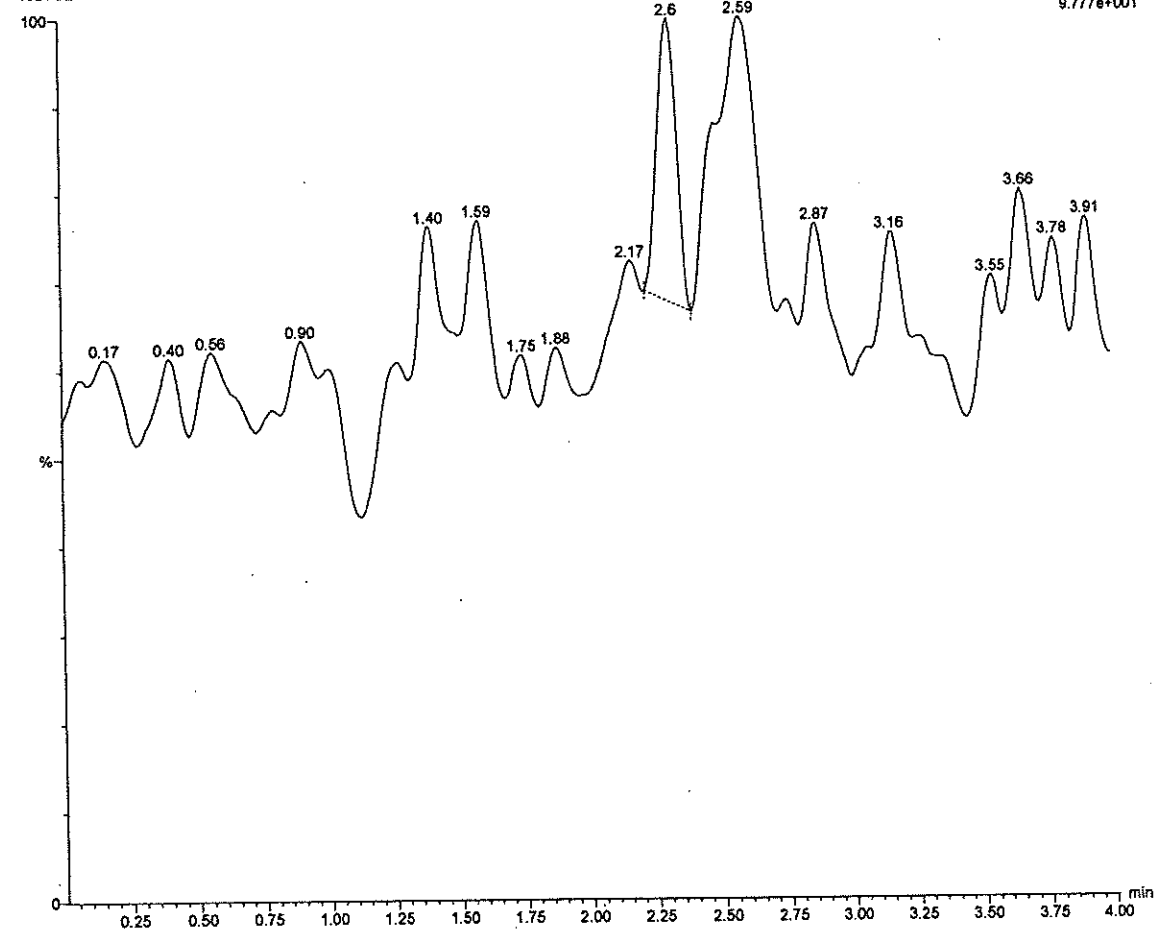
2.6

2.59

MRM of 1 channel, ES-

328.87 > 284.80

9.777e+001



	Name	RT	Area	Height
1	FRD 903			

Figure 19 Chromatograms of test fish analysis
day 21, Std. 0.02 mg/L (High concentration level)

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 11 May 2009 18:27:09

Date: 11-May-2009

Time: 16:43:39

Description: std 0.02mg/L

Name: S051109

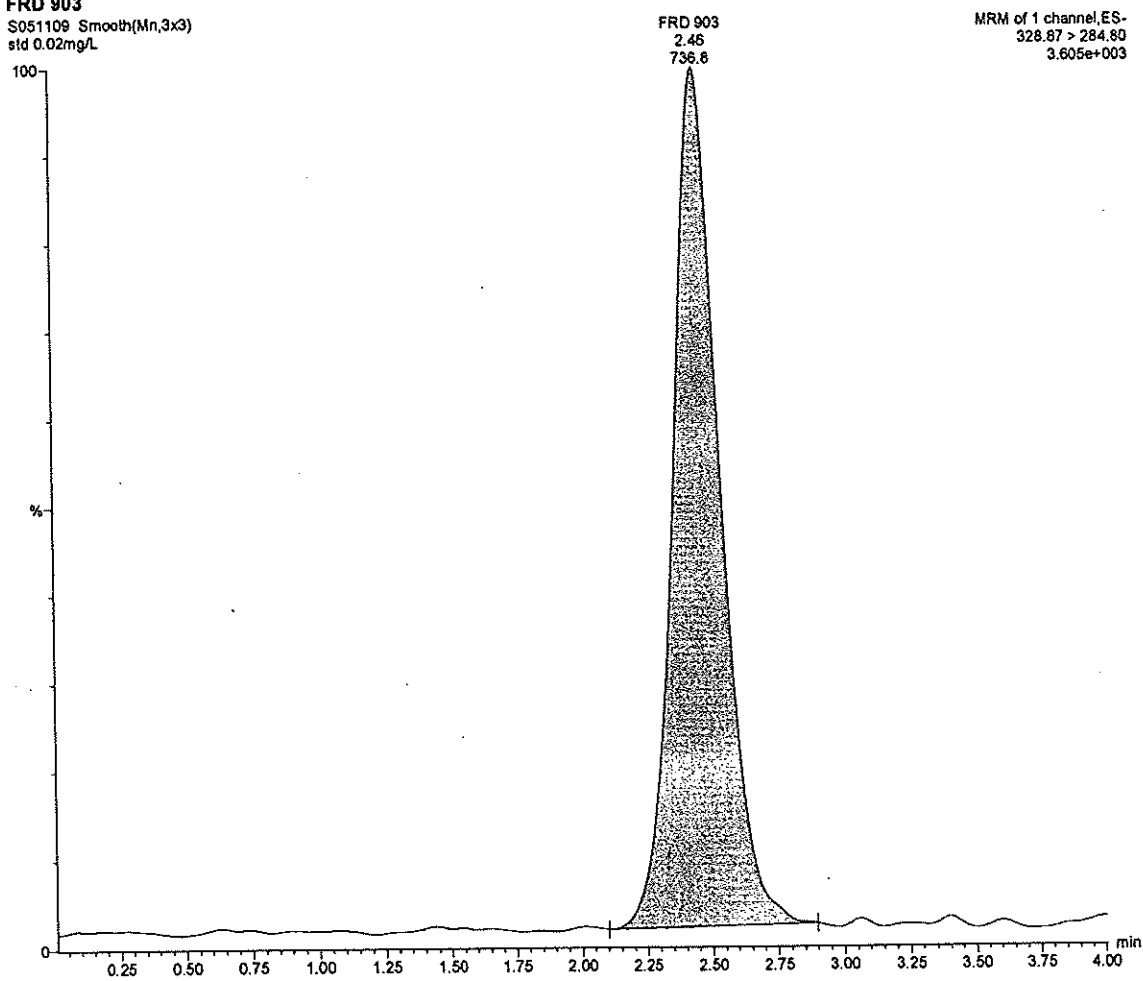
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQ\UDB\A080560_02

Vial: 1:A,4

User: 志

FRD 903

S051109 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.461	736.8	3525

Figure 20 Chromatograms of test fish analysis (continued)
day 21, High concentration level, No.1

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 11-May-2009

Time: 16:48:37

Description: 21DFH1

Name: 21DFH101

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,1

User: 志

FRD 903

21DFH101 Smooth(Mn,3x3)

21DFH1

FRD 903

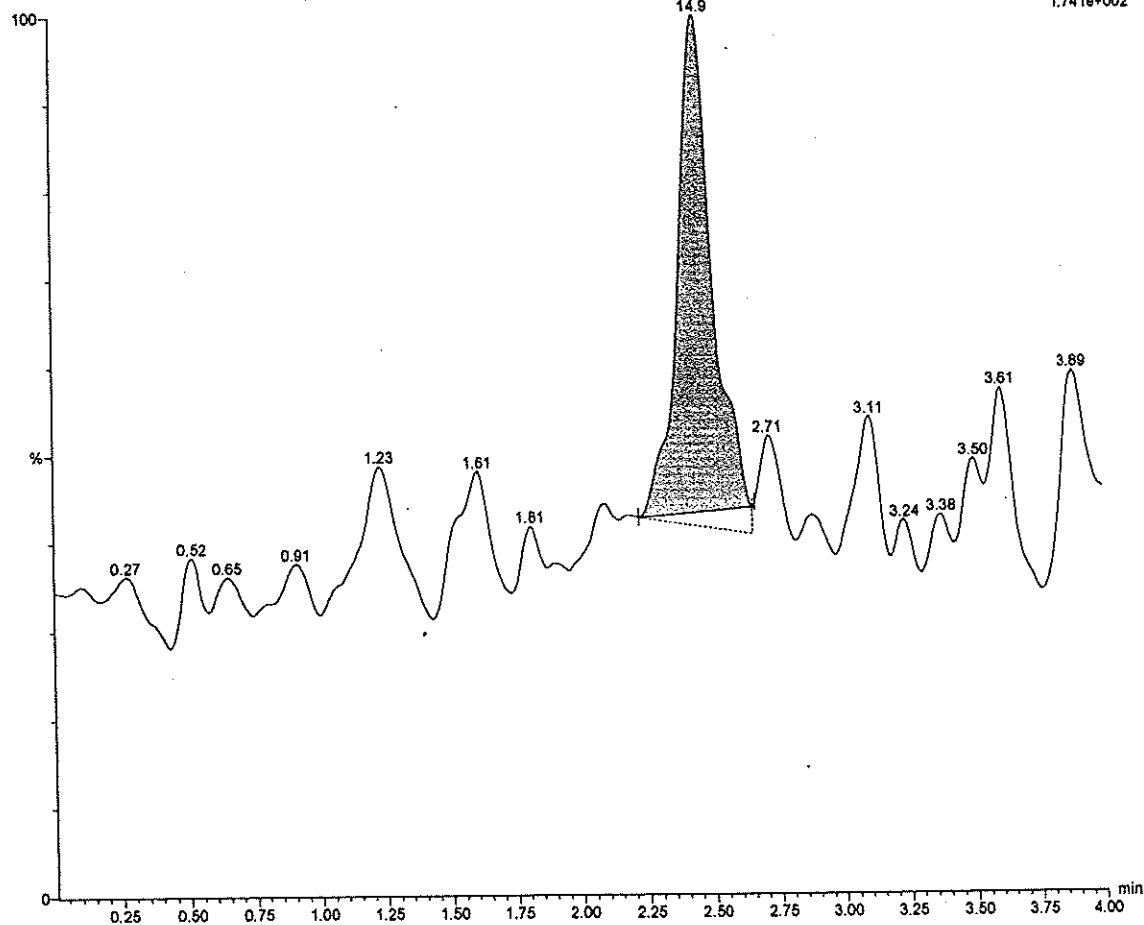
2.45

14.9

MRM of 1 channel, ES-

328.87 > 284.80

1.741e+002



#	Name	RT	Area	Height
1	FRD 903	2.452	14.9	99

Figure 21 Chromatograms of test fish analysis (continued)
day 21, High concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 11-May-2009

Time: 16:54:06

Description: 21DFH2

Name: 21DFH201

Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:B,2

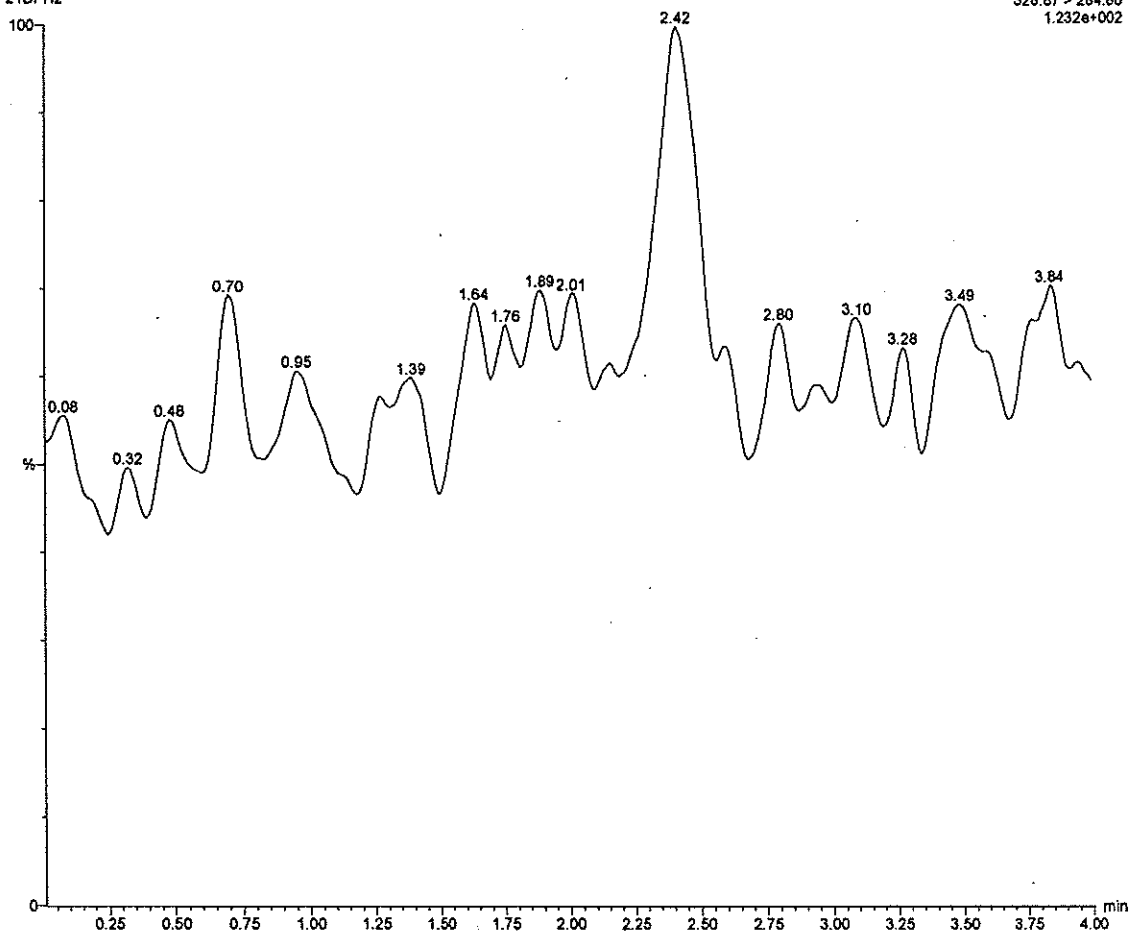
User: *ks*

FRD 903

21DFH201 Smooth(Mn,3x3)

21DFH2

MRM of 1 channel, ES-
328.87 > 284.80
1.232e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 22 Chromatograms of test fish analysis
day 21, Std. 0.02 mg/L (Low concentration level)

Quantify Sample Report MassLynx 4.1
A080560 FRD 903

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 11 May 2009 18:24:47

Date: 11-May-2009


Time: 16:59:38

Description: std 0.02mg/L

Name: S051110

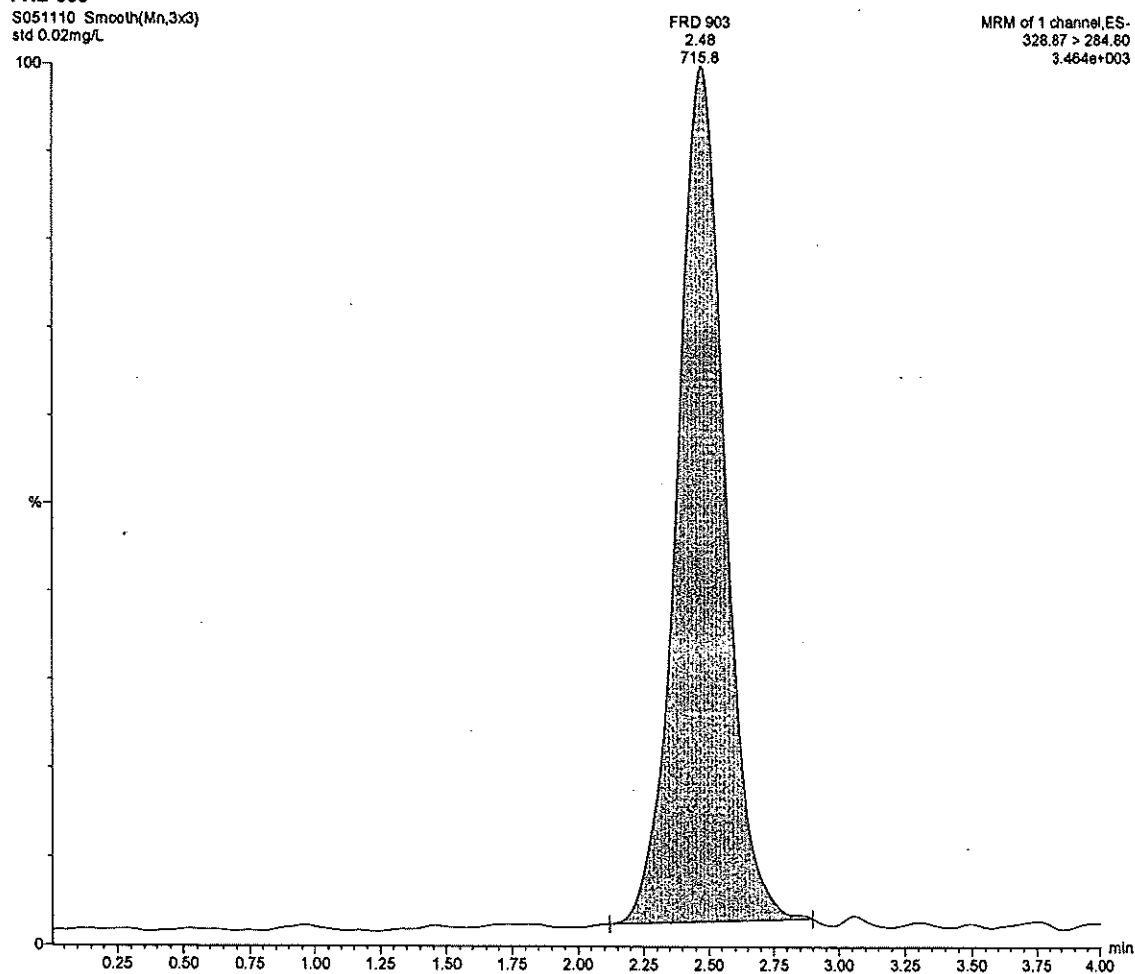
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,4

User: 

FRD 903

S051110 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.478	715.8	3368

Figure 23 Chromatograms of test fish analysis (continued)

day 21, Low concentration level, No.1

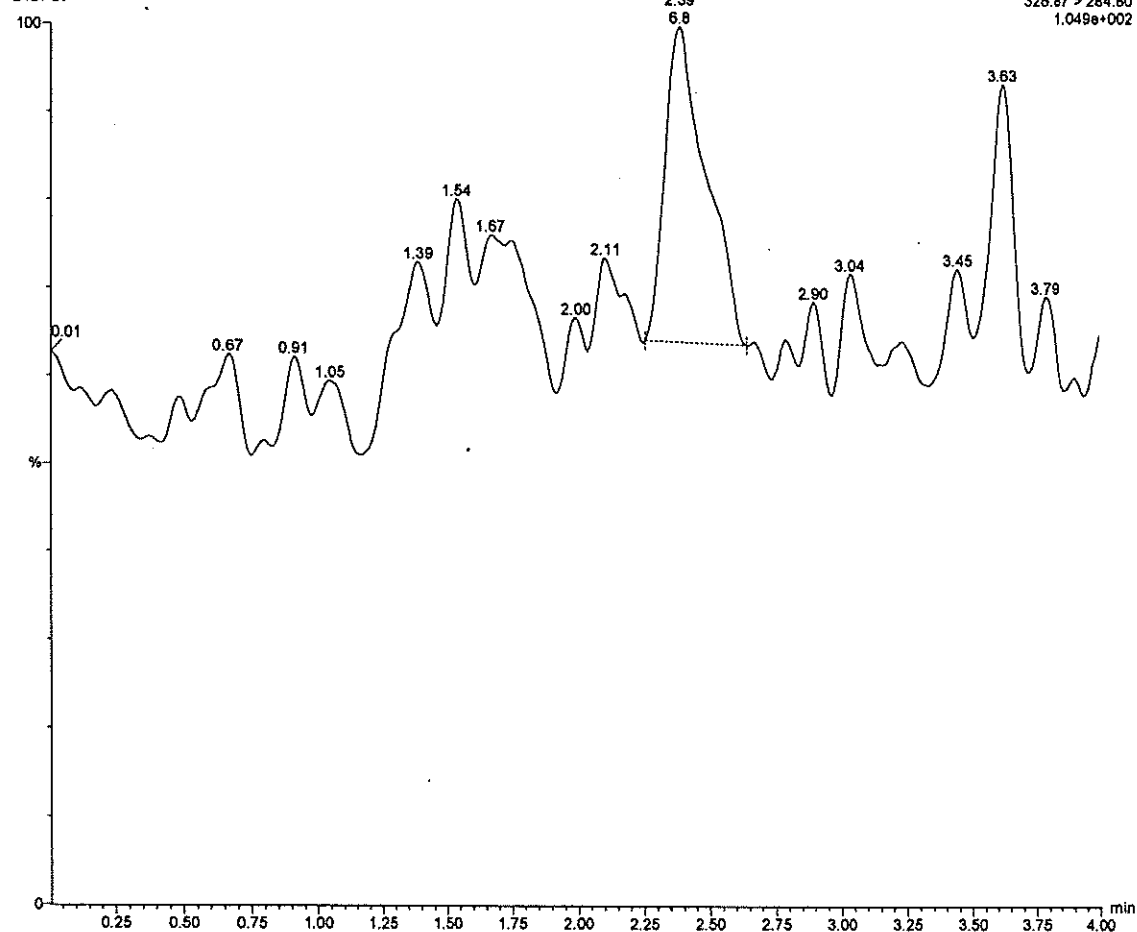
Quantify Sample Report
A080560 FRD 903**MassLynx 4.1**

Date: 11-May-2009**Time:** 17:05:13**Description:** 21DFL1**Name:** 21DFL103**Inlet Method Name:** C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02**Vial:** 1:A,6**User:** KS

FRD 903

21DFL103 Smooth(Mn,3x3)

21DFL1



	#	Name	RT	Area	Height
1	1	FRD 903			

Figure 24 Chromatograms of test fish analysis (continued)
day 21, Low concentration level, No.2

Quantify Sample Report
A080560 FRD 903

MassLynx 4.1

Date: 11-May-2009

Time: 17:10:43

Description: 21DFL2

Name: 21DFL203

Inlet Method Name: C:\MassLynx\bin\con\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,7

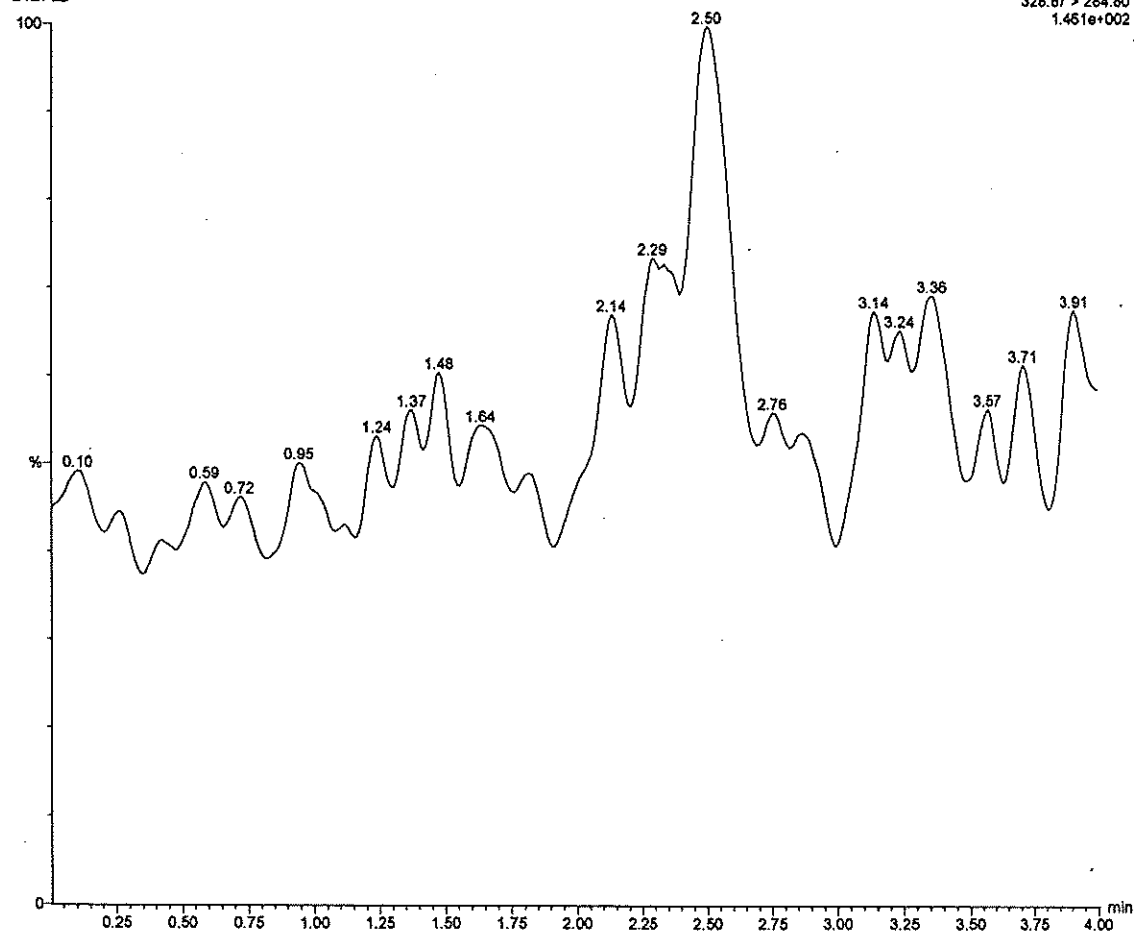
User: *KS*

FRD 903

21DFL203 Smooth(Mn,3x3)

21DFL2

MRM of 1 channel, ES-
328.07 > 284.80
1.451e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 25 Chromatograms of test fish analysis
day 28, Std. 0.02 mg/L (High concentration level)

Quantify Sample Report MassLynx 4.1
A080560 FRD903

Method: C:\MassLynx\blocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 14 May 2009 16:53:16

Date: 14-May-2009

Time: 16:15:12

Description: std 0.02mg/L

Name: S051409

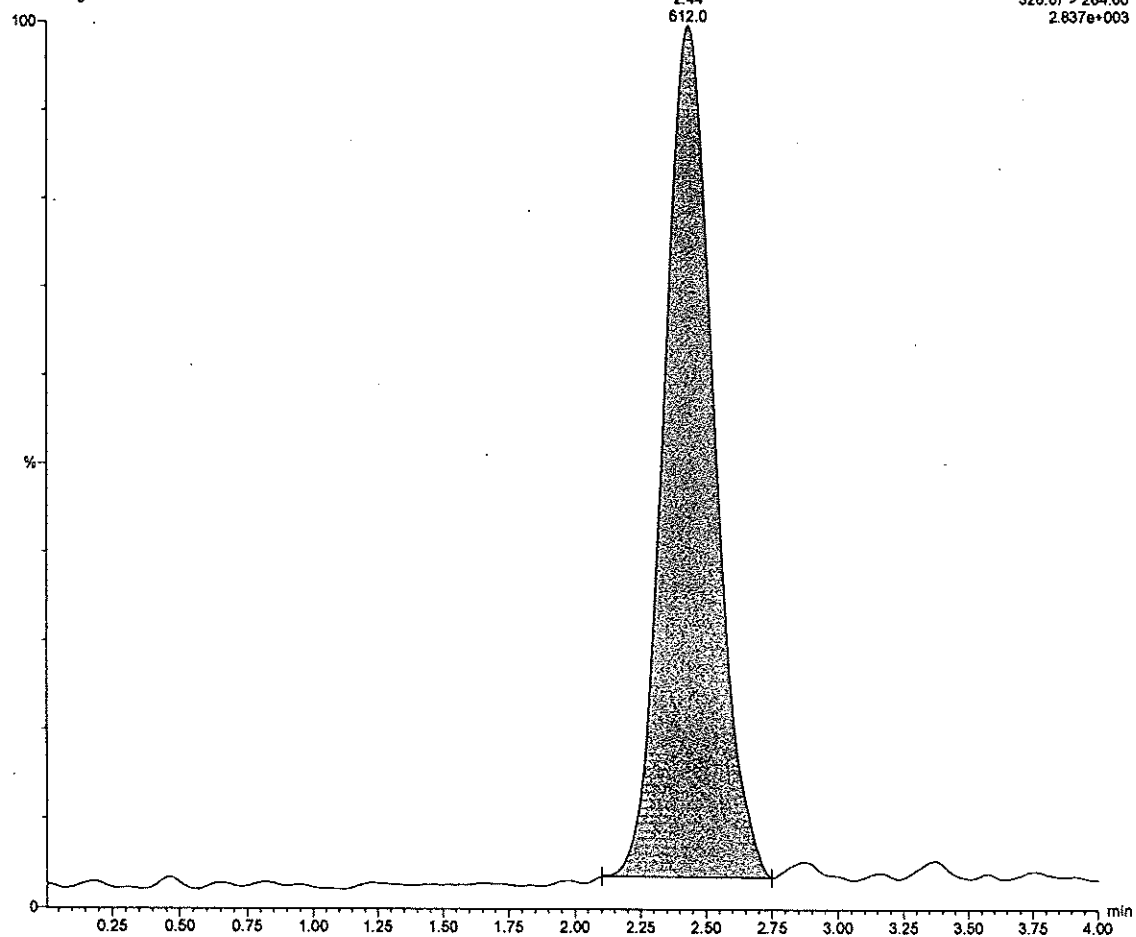
Inlet Method Name: C:\MassLynx\blocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: 志

FRD 903

S051409 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.444	612.0	2735

Figure 26 Chromatograms of test fish analysis (continued)

day 28, High concentration level, No.1

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 14-May-2009

Time: 16:20:10

Description: 28DFH1

Name: 28DFH1

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

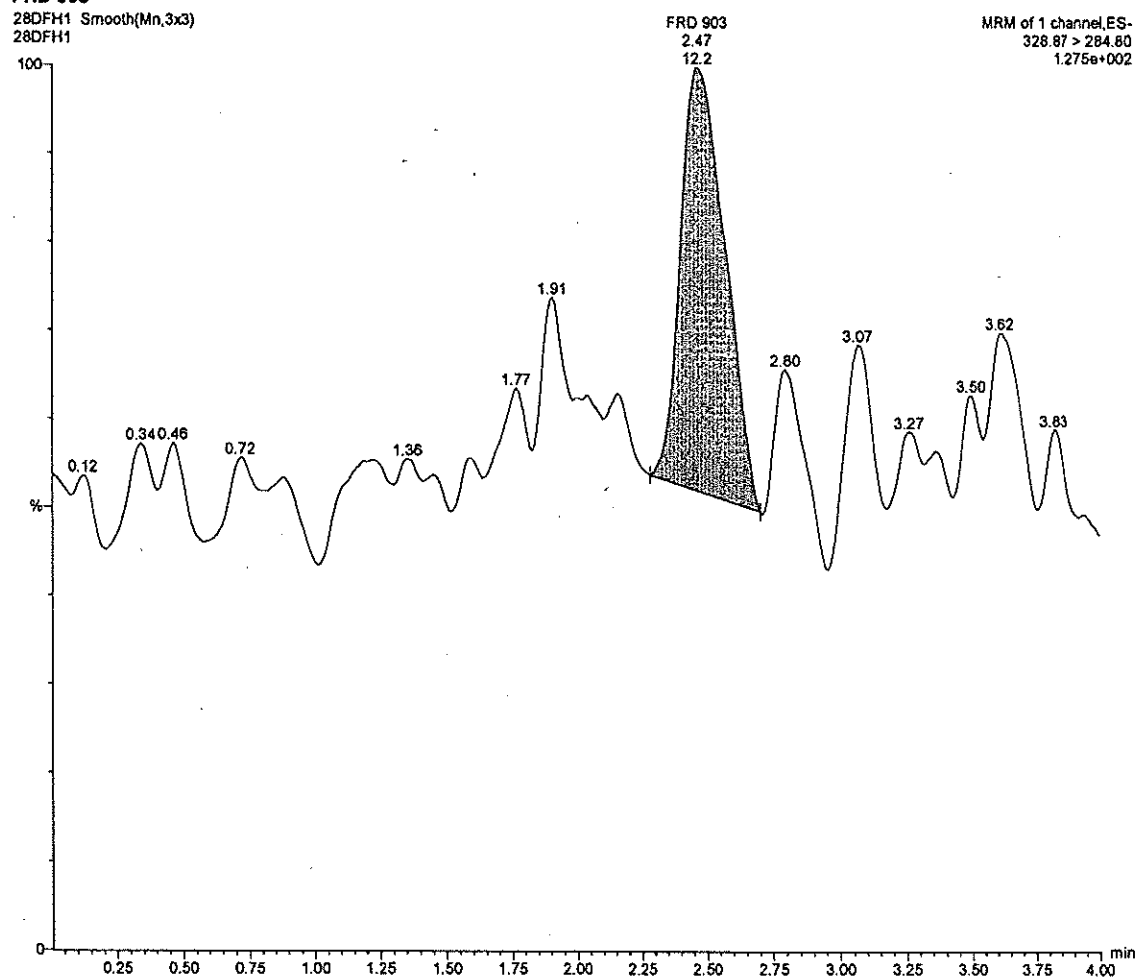
Vial: 1:B,1

User: 志

FRD 903

28DFH1 Smooth(Mn,3x3)

28DFH1



#	Name	RT	Area	Height
1	FRD 903	2.470	12.2	61

Figure 27 Chromatograms of test fish analysis (continued)

day 28, High concentration level, No.2

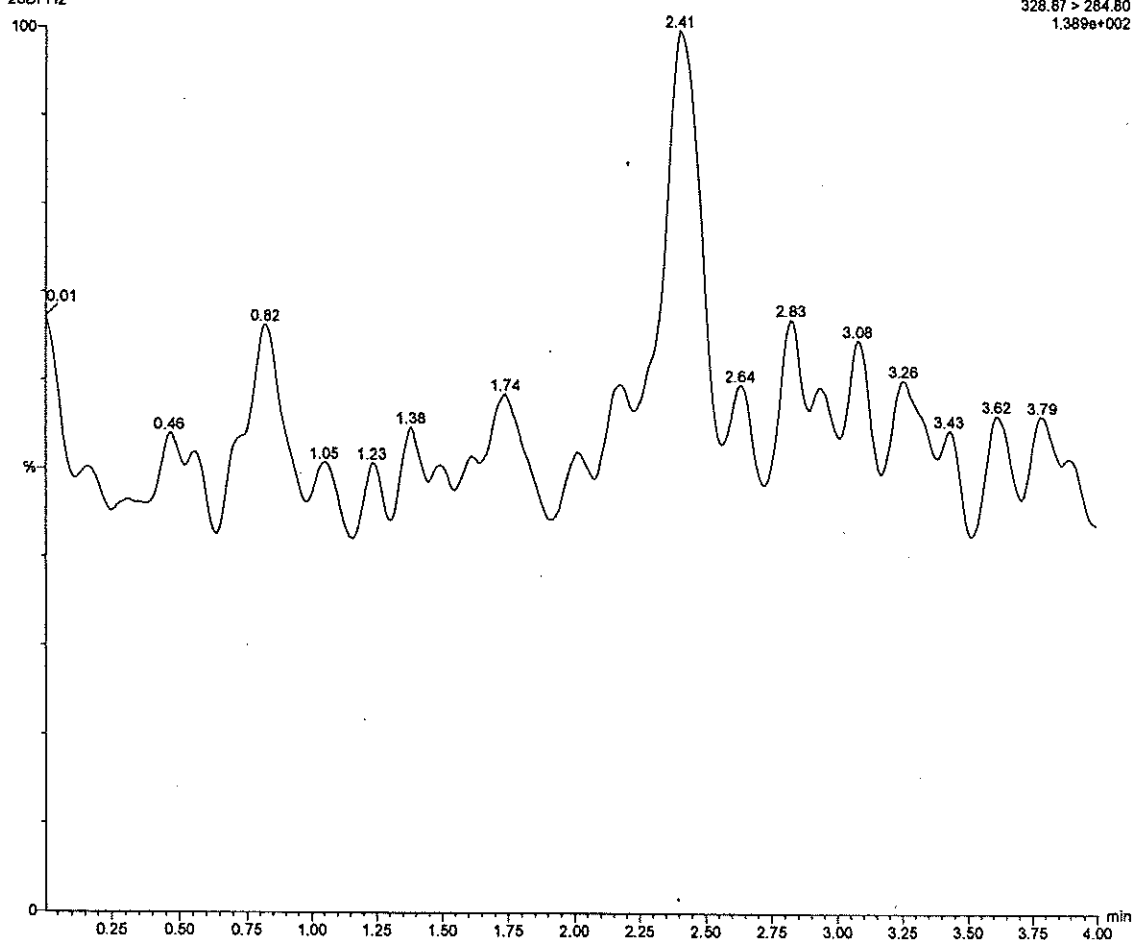
Quantify Sample Report MassLynx 4.1
A080560 FRD903

Date: 14-May-2009
Time: 16:25:39
Description: 28DFH2
Name: 28DFH2
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02
Vial: 1:B,2 志
User:

FRD 903

28DFH2 Smooth(Mn,3x3)
28DFH2

MRM of 1 channel, ES-
328.87 > 284.80
1.389e+002



#	Name	RT	Area	Height
1	FRD 903			

Figure 28 Chromatograms of test fish analysis
day 28, Std. 0.02 mg/L (Low concentration level)

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Method: C:\MassLynx\biocon\A080560 FRD 903.PRO\MethDB\FRD 903.mdb 01 May 2009 13:29:38
Calibration: 14 May 2009 16:52:53

Date: 14-May-2009

Time: 15:18:55

Description: std 0.02mg/L

Name: S051406

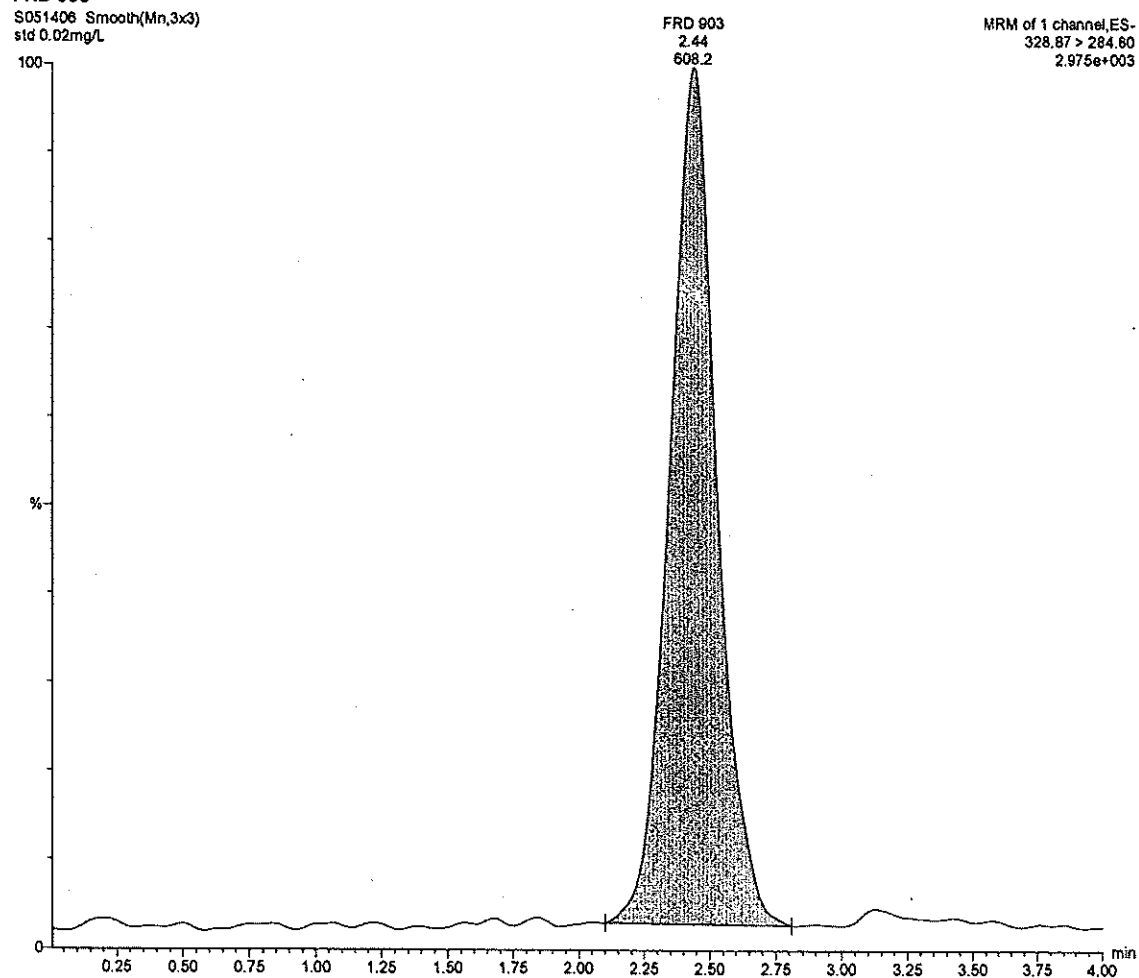
Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,3

User: 志

FRD 903

S051406 Smooth(Mn,3x3)
std 0.02mg/L



#	Name	RT	Area	Height
1	FRD 903	2.444	608.2	2889

Figure 29 Chromatograms of test fish analysis (continued)
day 28, Low concentration level, No.1

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 14-May-2009


Time: 15:23:53

Description: 28DFL1

Name: 28DFL1

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDE\A080560_02

Vial: 1:A,6

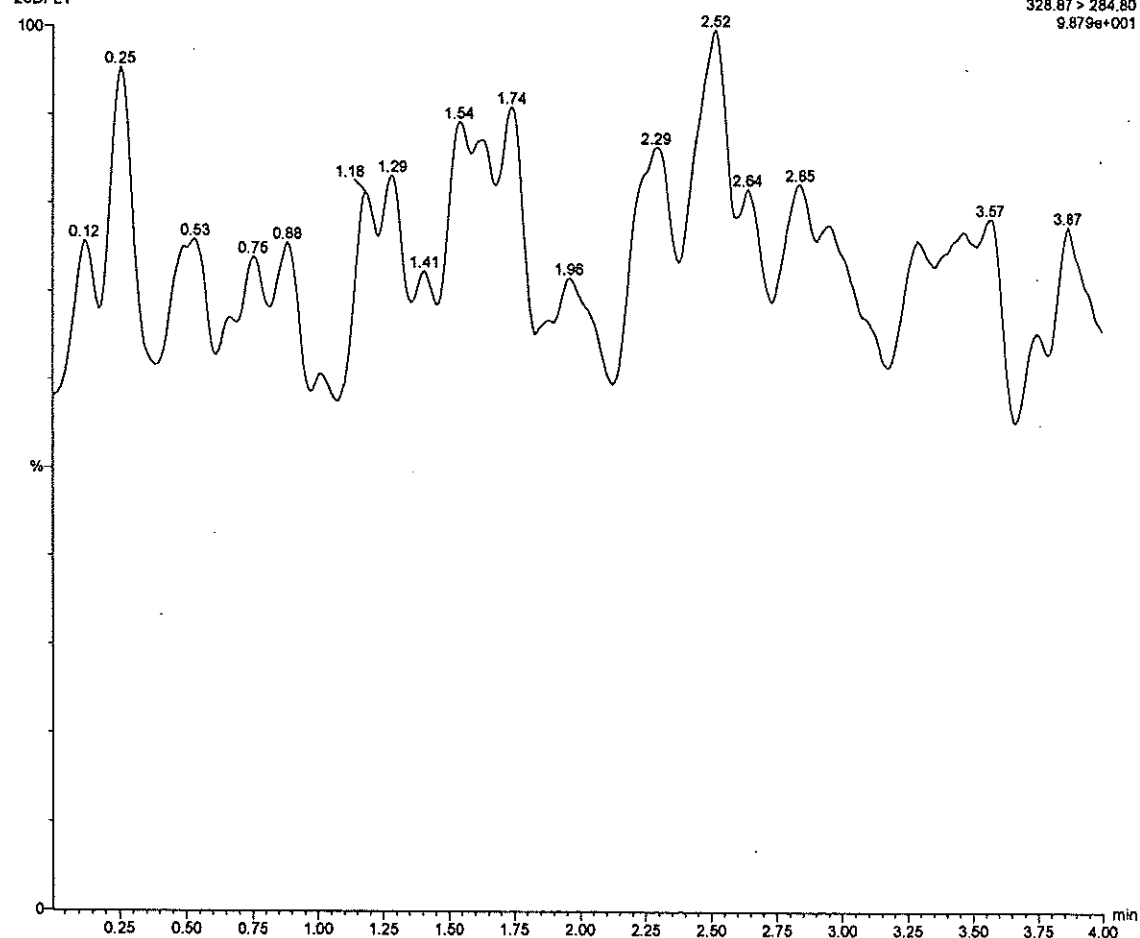
User: 

FRD 903

28DFL1 Smooth(Mn,3x3)

28DFL1

MRM of 1 channel, ES-
328.87 > 284.80
9.879e+001



#	Name	RT	Area	Height
1	FRD 903			

Figure 30 Chromatograms of test fish analysis (continued)
day 28, Low concentration level, No.2

Quantify Sample Report
A080560 FRD903

MassLynx 4.1

Date: 14-May-2009

Time: 15:29:24

Description: 28DFL2

Name: 28DFL2

Inlet Method Name: C:\MassLynx\biocon\A080560 FRD 903.PRO\ACQUDB\A080560_02

Vial: 1:A,7

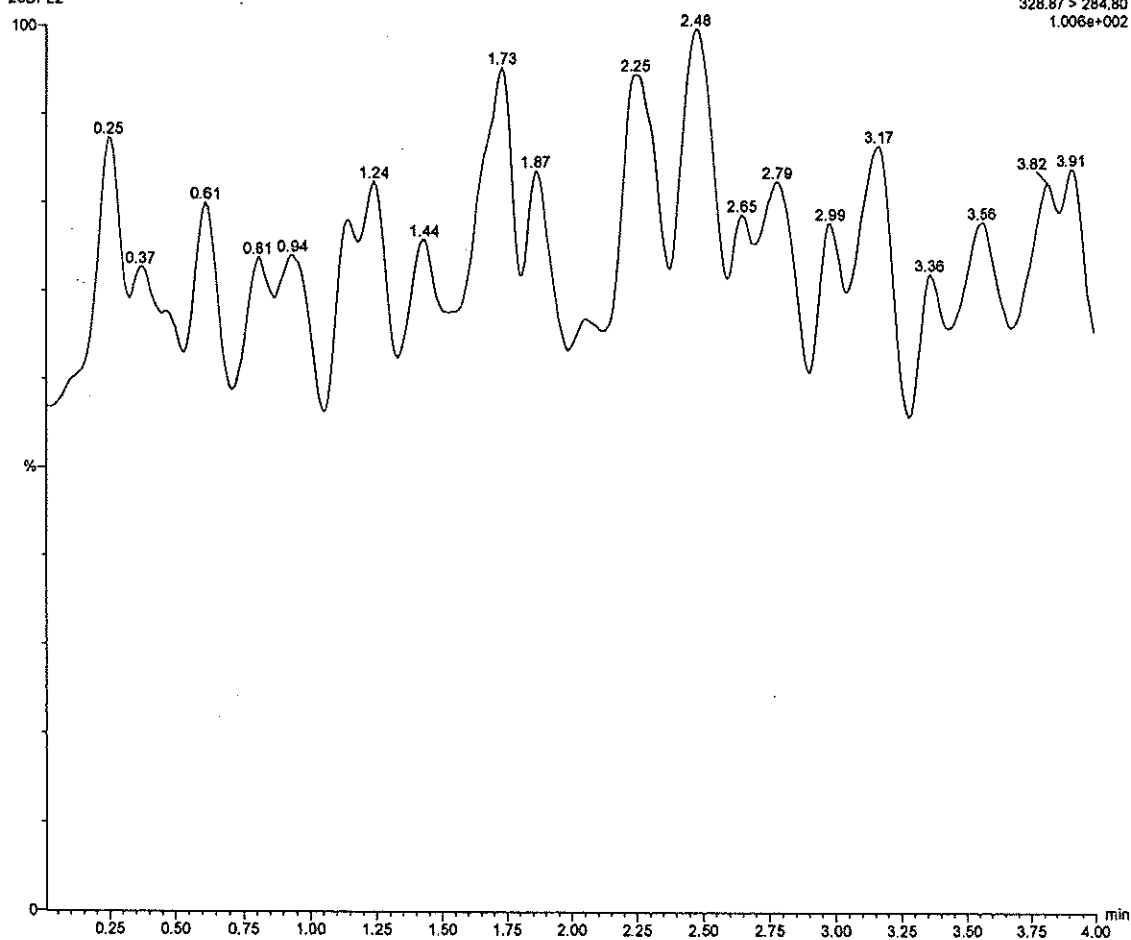
User: *KS*

FRD 903

28DFL2 Smooth(Mn,3x3)

28DFL2

MRM of 1 channel, ES-
328.87 > 284.80
1.006e+002



#	Name	RT	Area	Height
1	FRD 903			